LONG-TERM CONSEQUENCES OF ENVIRONMENTAL LEAD EXPOSURE IN KOSOVO: EFFECTS OF PRE AND POSTNATAL LEAD EXPOSURE IN EARLY ADULTHOOD

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Abstract

Long-term consequences of environmental lead exposure in Kosovo: Effects of pre and postnatal lead exposure in early adulthood

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Between May 1985 and December 1986, a cohort of 1,502 pregnant women was recruited at two government clinics in Kosovo (formerly a province of Yugoslavia) for a study of the relationship between environmental lead (Pb) exposure and birth outcomes. Subsequently, a representative group of 541 of their children were selected for long-term follow up. The children were followed longitudinally at six-month intervals for 12 years to examine the effects of environmental Pb exposure on a variety of health outcomes including cognitive and motor function, anemia, endocrine function and growth.   This work produced numerous landmark publications (Popovac et al, 1982; Graziano et al., 1990, 1991, 2004; Murphy et al., 1990; Factor-Litvak et al, 1993, 1996, 1998, 1999; Wasserman et al,. 1992, 1994, 1997, 1998, 2000) that contributed to the modification of environmental policies to reduce Pb exposure worldwide. The long-term study ultimately linked environmental Pb exposure from the Trepca mining and smelting operations in Mitrovica to adverse effects on intelligence, motor function, blood pressure, renal, endocrine and hematological functioning. Follow up rates over time were excellent in that 70% of the total cohort was evaluated at 6 years of age, and 65% were evaluated at 12 years of age, at which point the study was - until now - concluded.

For the present study, we located 101 members of the original study cohort and requested their participation in a follow-up study in which participants were evaluated to assess their current blood lead (BPb) levels and health outcomes as follows: a) blood pressure; b) biomarkers

of endothelial cell function that are associated with cardiovascular disease; c) and measurements of erythropoietin, a glycoprotein hormone produced in the kidney that regulates the production of red blood cells in the bone marrow.   The participants, whose environmental exposure history is very well documented from 12 weeks of gestation through 12 years of age, were between 25-26 years of age during the follow-up study.

We found a statistically significant association between BPb and systolic blood pressure (sBP), and a marginally significant association between BPb and diastolic blood pressure (dBP), which is consistent with a multitude of studies and meta-analyses referenced in this dissertation. These results provide further evidence that recent circulating dose, as estimated by BPb, or as estimated by lifetime cumulative exposure, is associated with slight increase in sBP. Furthermore, we detected a suggestive relationship between BPb and levels of circulating serum intercellular adhesion molecules (sICAM-1) and serum intravascular adhesion molecules (sVCAM-1), possibly a mechanism by which Pb may lead to increased BP. These findings support the hypothesis that the exposure to Pb either prenatally or in early adulthood, may lead to increased BP and increased circulating levels of sICAM-1 and sVCAM-1 later in life. Lastly, the results regarding the serum erythropoietin (EPO) production presented here resemble the findings reported in this cohort at 4.5 and 6.5 years of age and in contrast with the findings in this cohort when the study participants were 9.5 and 12 years of age (Graziano et al., 2004). In addition, they also contrast the findings reported in the anemic mothers of this study cohort (Graziano et al., 1991) where serum-EPO levels were lower in those with higher BPb levels.

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ABBREVIATIONS

ALAD	δ-Aminolevulineate dehydratase
ALM	Adult Lead Methodology
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
As	Arsenic
BMI	Body mass index
BRHS	British Regional Heart Study
BUN	Blood urea nitrogen
BPb	Blood lead
BP	Blood Pressure
CDC	US Centers for Disease Control
CVD	Cardiovascular disease
CKD	Chronic kidney disease
dBP	Diastolic blood pressure
ELIZA	Enzyme linked immunosorbent assay
EP	Erythrocyte protoporphyrin
EPA	US Environmental Protection Agency
EPO	Erythropoietin
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometer
GFR	Glomerular filtration rate
Hgb	Hemoglobin
NAG	N-acetyl-b-D-glucosaminidase
NHANES	National Health and Examination Surveys
OSHA	US Occupational Safety & Health Administration
Pb	Lead
PCT	Proximal convoluted tubules
ROS	Reactive oxygen species
sBP	Systolic blood pressure
SCr	Serum creatinine
sICAM-1	Serum intercellular adhesion molecules
sVCAM-1	Serum intravascular adhesion molecules
VDR	Vitamin D receptor
WHO	World Health Organization
XRF	X-ray fluorescence
ZPP	Zinc protoporphyrin

ACKNOWLEDGEMENTS

I dedicate this to my children in hopes of inspiring them to follow their dreams and to work hard achieving them; and my long departed parents who always inspired me.

CHAPTER I: SPECIFIC AIMS AND HYPOTHESES OF THE STUDY

The review of the literature for this dissertation study has shown inconsistencies and missing links between environmental Pb exposure (pre-and postnataly and in early adulthood) and health outcomes later in life. Additional studies in populations with a wide range of exposures are necessary in order to better elucidate dose-response relationships between Pb and its effect of BPb on blood pressure, as well as on kidneys and hemopoietic system. Although the effects of Pb on blood pressure are well documented, we have not come across any studies, prospective in a nature, with a wealth of early documented exposure and follow-up in adulthood. The studies that are the basis for this dissertation will try to address the inconsistencies that we have identified making use of the data from this population based on this longitudinal study. This study differs from other studies in the following ways: BPb, EPO, Hemoglobin (Hgb), and blood pressure (BP) measurements were obtained numerous times between birth and age of 12.5 and again at the age of ~ 24-25 years. The fact that we have a full complement of data from birth through 12.5 years of age, and again at 24-25, sets this cohort apart from many other cohorts. In addition, the wide range of BPbs in the studied population enables a better assessment of a dose-response relationship. When examining associations between health outcomes and Pb exposure, we and others have typically used alternative ways of determining the most critical period of exposure. Of course, we will examine the association with concurrent BPb. However, we will also examine the associations between health outcomes and the total area under the BPb vs. age curve (AUC) (using the trapezoidal method), for pre-natal and AUC for early post-natal periods (birth to age 2, 2-4, 4-7, and 7-12 years old). In this manner we will attempt to determine if there

is a critical time period of exposure with regard to each health outcome. The study will have the following specific aims and hypotheses listed below:

1.1. Specific Aim 1

Using the baseline cross-sectional information of the previous study and current cohort, our first aim was to examine the association between environmental Pb exposure and blood pressure and cardiovascular health at age ~ 24-25 years. Pb exposure was estimated using past measurements of BPb which were already available from birth through age of 12, as well as a new concurrent measurement.

We will also examine the impact of environmental lead exposures on the cardiovascular system by examining the association between BPb over time manifested by changes in the circulating levels of two biomarkers of endothelial cell functioning, namely soluble intercellular adhesion molecule (sICAM-1) and soluble vascular cell adhesion molecule (sVCAM-1). This specific aim was developed to test the following hypothesis:

    i. **Hypothesis 1:** Higher levels of prenatal and early adulthood Pb exposure are associated with an increase in blood pressure in adulthood.

    ii. **Hypothesis 2:** Higher levels of prenatal and early adulthood Pb exposure are associated with increased levels of the circulating sICAM-1 and sVCAM-1 in adults.

1.2 Specific Aim 2

Using the baseline cross-sectional information on child participants of the previously described prospective study, we aimed to assess the association between cumulative Pb exposure and its effect on renal endocrine function by measuring serum EPO. This specific aim was developed to test the following hypothesis:

i. **Hypothesis 3:** Higher levels of prenatal and early adulthood Pb exposure are associated with a decline in the slope of the relationship between EPO and BPb with age (after adjusting for Hgb), suggesting that individuals with elevated BPb early in life have a decreased capacity to produce EPO over time.

CHAPTER II: INTRODUCTION AND LITERATURE REVIEW

2.1 Background and Significance

In the field of environmental health, exposure to Pb has remained one of the most studied topics. However, even with persistent research on this topic, and continued efforts to reduce exposure, long term effects of Pb on public health still require more *adequate* attention. More specifically, analyses of relationships between prenatal and early childhood Pb exposure and blood pressure and effects on erythropoietin production later in life are not adequate. A significant study undertaken in two Kosovo towns beginning in the mid 1980s shed much light on these topics, and produced numerous landmark publications (Popovac et al, 1982; Graziano et al., 1990, 1991, 2004; Factor-Litvak et al,. 1993, 1996, 1998, 1999; Wasserman et al,. 1992, 1994, 1997, 1998, 2000) that contributed to the implementation of environmental policies. The research studies cited in this dissertation examine the relationship between prenatal and early childhood exposure to environmental Pb and increases in blood pressure and effects on EPO production. As background and introduction to these studies, this section provides an extensive literature review concerning the effects of Pb exposure on blood pressure and renal function as measured by EPO production.

The town of Mitrovica with its 115,000 inhabitants is located in a mountainous region of Kosovo (part of Yugoslavia until Kosovo's independence in 2008) and approximately 40 km from the capitol city of Prishtina. Until recently, the region's industry was dominated by the "Trepca Lead Mines and Smelter" located on the outskirts of the town of Mitrovica. At the height of production, it employed up to 23,000 workers and was one of the biggest companies in the former Yugoslavia, as well as one of the largest Pb smelters in Europe. In terms of air

pollution, Pb dust was the most abundant pollutant in the region; in 1979, 5,756 kg of Pb dust were emitted daily from the Pb smelter alone. The air-Pb concentrations ranged from 21.3-29.2 $\mu g/m^3$ in the mid 1980s (Popovac et al., 1982). This was 100 times greater than allowable limits of air-Pb concentrations in the former Yugoslavia.

Early studies on this topic attempted to determine the effects of these emissions on blood-lead (BPb) and erythrocyte protoporphyrin (EP) levels and to evaluate the health impacts of Pb exposure. To that end, a small pilot study was conducted in 1978 which suggested that the incidence of elevated blood-lead in children was substantial (Popovac et al., 1982).   Between 90 and 95 percent of children ages 0-15 years old had BPb levels ranging from 30-70 µg/dL or greater (In the 1970's, the "normal or safe levels" for BPb were considered to be at 60 µg/ dL; this level was reduced by the US Centers for Disease Control (CDC) to 30 µg/ dL in 1980, to 25 µg/ dL in 1985, to 10 µg/ dL in 1991, and to 5 µg/ dL in 2012). In addition, a follow-up survey was conducted to obtain a *more representative sample* of BPb in Mitrovica in 1980 (Popovac et al., 1982). Table 2-1 below shows the increasing trend of erythrocyte protoporphyrin (EP) between 1978 and 1980. This trend was believed to be associated with the increase in the mean air Pb concentrations measured at five different locations in the city (Popovac et al., 1982).

Non-exposed Controls, 1978			Exposed Area Residents, 1978				Exposed Area Residents, 1980		
EP	B-Pb	N	EP	N	B-Pb*	N	EP	B-Pb	N
Age (Yr)	(µg/g Hgb)	µg/dL		(µg/g Hgb)		µg/dL		(µg/g Hgb)	µg/dL	
0-3	3.9	6.8	37	8.0	104	37.4	57	9.6	46.3	92
3-5	2.1	7.5	10	6.3	25	45.1	11	8.6	44.4	56
3-10	1.5	8.7	19	6.1	56	50.9	20	8.6	45.9	37
10-15	1.4	8.7	6	4.7	38	44.6	10	8.7	46.8	56
>15	0.9	10.0	1	3.6	97	27.6	9	5.3	35.3	138
			73		104					379

Table 2-1: NOTE: The mean EPs for the cases where BPb was determined were 12.0, 10.9, 11.6, 8.6, and 8.1 respectively, youngest to oldest. Table 1: Erythrocyte Protoporphyrin (EP) Concentrations and Blood Lead (B-Pb) Concentrations of Residents of Two Cities in Kosova; Source: Popovac et al., Archives of Environmental Health, 1982, Volume 37, 19-23).

Subsequent to the findings of the these two surveys, a prospective cohort was established in order to adequately evaluate the health effects of these levels of exposure, first during pregnancy, and later, during the growth and development in early childhood. In order to include participants with a wide range of Pb exposure, residents in the non-exposed city of Prishtina were also recruited. Initially, 1,502 pregnant women were recruited at two government clinics for a study of the relationship between environmental Pb exposure and birth outcomes between May, 1985 and December 1986 (Graziano et al., 1990). As indicated in Figure 2-1 below, the mean mid-pregnancy BPb levels in women recruited in Mitrovica were much higher than in women recruited in Prishtina (Graziano et al., 1990).
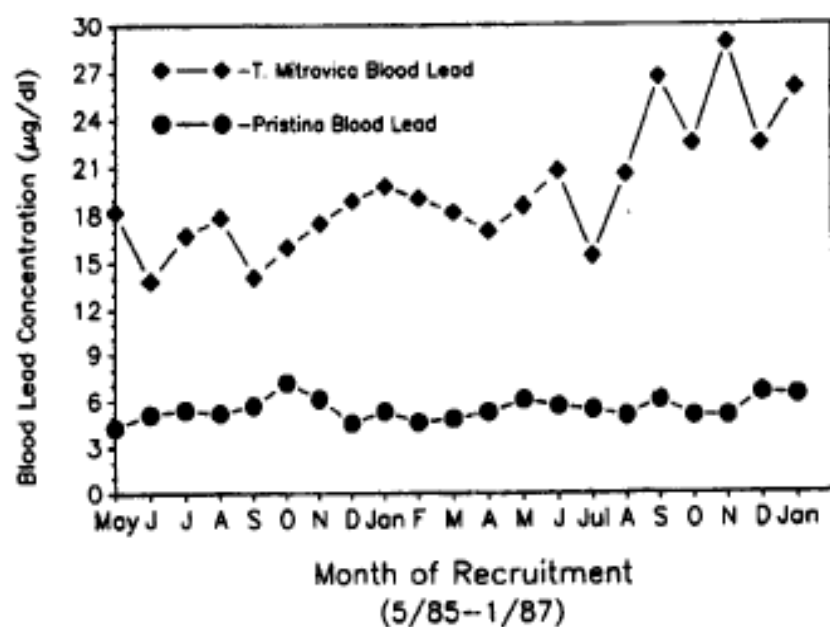
Figure 2-1: Mean mid-pregnancy BPb (µg /dl) for women recruited into the study during the months of May 1985 through January1987. Each point represents a different group of women, i.e., those recruited during that month; Source: Graziano et al., Environmental Health Perspectives, 1990, Volume 89, 95-100.

Elevated EP concentrations are considered to be indicative of Pb toxicity and/or iron deficiency (Graziano et al., 1990). Hematologic findings in children in both towns revealed that serum ferritin concentration (Figure 2-2), a measure of the adequacy of tissue iron stores, was

slightly higher in Mitrovica than in Prishtina (Wasserman et al., 1992). Despite the higher iron

stores, the mean EP was elevated in Mitrovica after the 6th month of age. Since the hemoglobin

levels were also similar, with no apparent difference in iron stores, these findings reflected some

degree of metabolic inhibition of red cell heme synthesis due to lead (Graziano et al., 1990). It is

important to note that EP concentrations have an exponential relationship with BPb levels only

after reaching a BPb threshold of 15-18 µg/dL, at which point they start to rise, giving the dose-

response relationship the shape of a "hockey stick" (Piomelli et al., 1982).
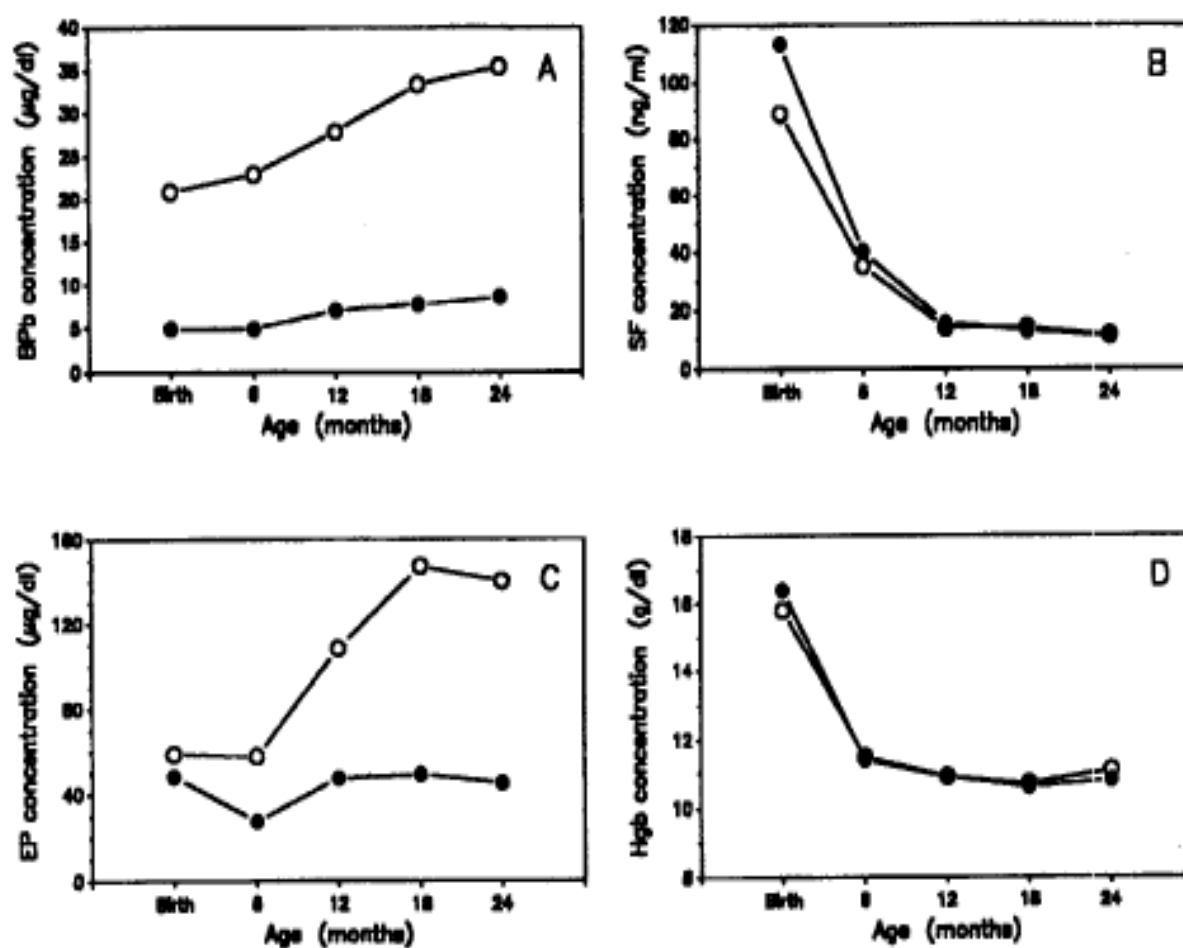
Figure 2-2: Hematologic finding for children in K. Mitrovica (clear circles) and Prishtina (dark circles) who were assessed at 24 months of age. A, Geometric mean BPb concentrations. B. Geometric mean ferritin concentrations. C. Geometric mean EP concentrations. D. Geometric mean hemoglobin concentrations. SF Serum Ferritin; Hgb, hemoglobin. Source: Wassermann et al., The Journal of Pediatrics, Volume 121, Part 1, 1992

As a result of regional wars of the last 15-20 years, there has been a significant reduction of the "Trepca Lead Mines and Smelter" capacity and output. Consequently, environmental Pb exposure in the town of Mitrovica decreased beginning around 1998, though air Pb measurements are not available.

2.2 Lead Exposure and Metabolism

Harmful effects of exposure to Pb have been recognized and documented for centuries. Known as one of the metals of antiquity, Pb has been mined and used by many generations in a wide array of applications. Its wide use peaked during the industrialization of the developed world and it continues to be ubiquitous environmental contaminant in the developing world. Although naturally occurring in the environment, human activities are the main reason for high levels found throughout the environment. Whether in the home environment or occupational setting, there are many ways in which exposure to Pb occurs: through air, drinking water, food, contaminated soil, deteriorating paint, and dust. As a result of a significant body of scientific work, there has been a concerted effort to reduce exposure to this harmful pollutant. Unfortunately, that has not been the case in much of the developing world. Pb can be absorbed in a number of ways, i.e., through the gastrointestinal tract as well as through lungs and skin (Figure 2-3) (Rabinowitz et al., 1976 in EPA, 2001). Pb absorption from the gastrointestinal tract depends on several factors, including particle size, gastrointestinal transit time, nutritional status and age (Greenberg et al, 1986). The smaller the particle size, the greater the absorption. Thus, exposure to Pb dust results in higher absorption than exposure to the equivalent amount of lead from chips of Pb paint (Greenberg et al., 1986).

Nutritional status also affects the absorption of Pb. Iron and calcium deficiencies, low-energy (calorie) intake, and high-fat intake have been associated with enhanced Pb absorption.

While 10-15 percent of ingested Pb is absorbed into the bloodstream, as much as 90 percent is absorbed after inhalation (Greenburg et al., 1986). In addition to being largely bound to erythrocytes in the blood, Pb accumulates in soft tissues and bones (Rabinowitz 1976 in EPA, 2001). Pb has a half-life of between 20-40 days in blood, and 30-40 days in soft tissue (Barry et al., 1981). However, a longer half-life of Pb in the blood stream of Pb workers has been reported with the Pb body burden being a determining factor (Barry et al., 1981, Kang et al., 1983). Bone contains about 90 percent of the total body Pb content. Children absorb Pb more readily than adults (Greenberg et al., 1986; Alexander, et al., 1974; Ziegler et al., 1978). While adults absorb 10 to 15 percent of the ingested quantity, this amount can increase to 50 percent in infants, young children and pregnant women (Rabinowitz et al., 1980; Watson et al., 1986; Markowitz et al., 2000,). Absorption through the gut is the predominant route for children and Pb bioavailability increases when dietary intakes of iron, calcium, phosphorus, or zinc are low (Rabinowitz, et al., 1980; Markowitz et al., 2000,).

Pb that diffuses into bone is stored there for a period that corresponds to a half-life of several decades (Rabinowitz et al., 1976 in EPA, 2001, Rosen et al., 1993). Bone calcification is most active in trabecular bone (e.g., patella) during infancy and childhood. However, during adulthood, calcification occurs mostly in cortical (e.g., tibia) and trabecular bone (Aufderheide and Wittmers, 1992). As a result of high bone formation rate in early childhood, a rapid uptake of circulating Pb into mineralizing bone occurs. However, since during this stage bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorbtion rate (O'Flaherty et al., 1995), much of the Pb acquired early in life is not permanently fixed in the bone. Bone Pb tends to increase with age and is mostly stored in dense bones such as tibia, with a half-life of 10-30 years (Rabinowitz et al., 1976; Barry et al, 1981; O'Flaherty et al., 1995).

Increased bone turnover during pregnancy, menopause, lactation, or immobilization can increase BPb levels. Most of the Pb that is excreted is eliminated via renal glomerular filtration and tubular secretion (approximately 75 percent). Other routes of excretion include bile, gastrointestinal secretions, hair, nails and sweat. Generally, Pb excretion is low, with the most significant route being via the urinary tract. Although minute amounts of Pb are excreted through the sweat and the nails, these routes do not have any practical significance.
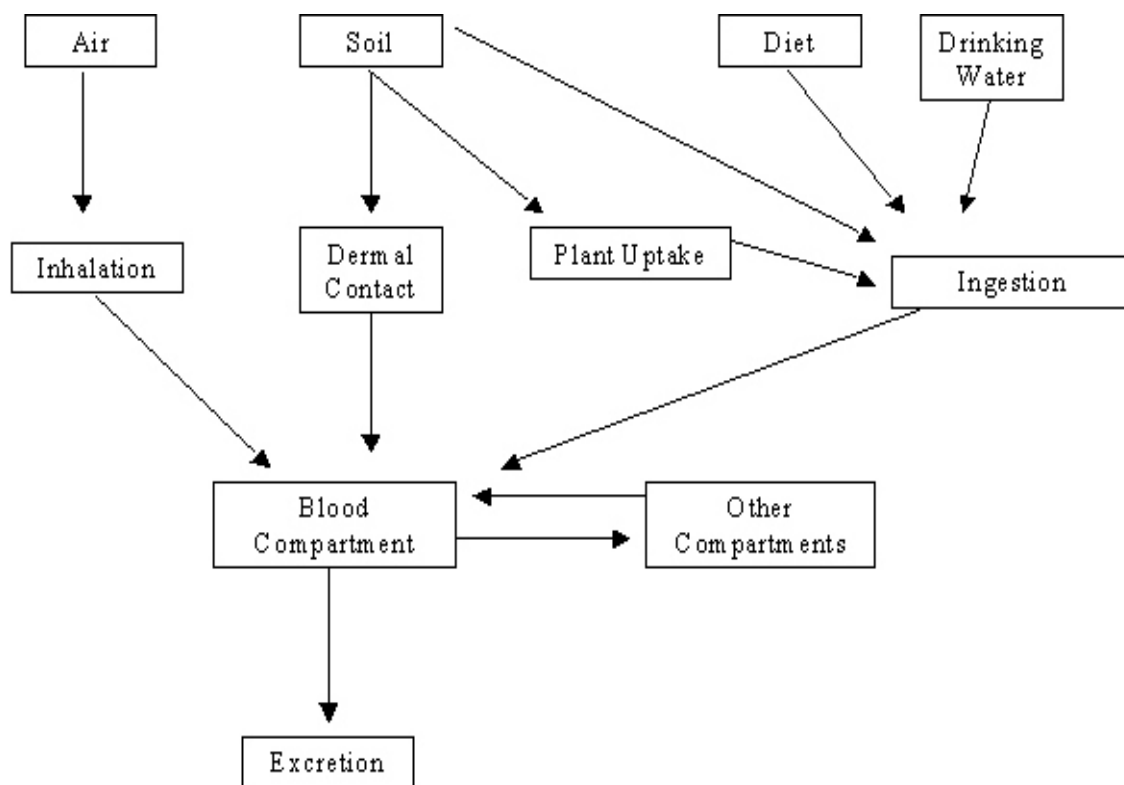
Figure 2-3: Rabinowitz et al. Metabolism Model for Lead, 1976; Review of Adult Lead Models Evaluation of Models for Assessing Human Health Risks Associated with Lead Exposures at Non-Residential Areas of Superfund and Other Hazardous Waste Sites Final Draft: August 2001. Source: EPA, Adult Lead Risk Assessment Committee of the Technical Review Workgroup for Lead (TRW).

2.3 Description of Lead Toxicokinetics and Toxicity

BPb concentrations vary considerably with age, physiological state (e.g., pregnancy, lactation, menopause), and numerous factors that affect exposure. As mentioned above, Pb in blood is found primarily (~99 percent) in the red blood cells (Bergdahl et al., 1997b, 1998; Hernandez-Avila et al., 1998; Schutz et al., 1987). Most of the Pb found in red blood cells is bound to proteins within the cell rather than the erythrocyte membrane (Bergdahl et al., 1997b, 1998).   In their thorough review of Pb biokinetics, the US Environmental Protection Agency (EPA) described a number of models that simulate the changes in Pb concentrations in various compartments. EPA details the work of Rabinowitz and his colleagues who in 1976 introduced a model that simulates changes in BPb concentrations in adult males in response to Pb uptake. Their model was based on data collected from five healthy subjects who received oral doses of stable Pb-isotopes for various periods of time. The model includes three compartments representing kinetically distinct Pb pools in the body (Figure 4) (Rabinowitz, 1976 in EPA, 2001). "Pool 1" represents the central compartment, i.e., blood and other extra-cellular fluids that rapidly equilibrate with whole blood. It contains approximately 1 percent of total Pb body burden at a given time and it has the shortest half-life (30±5 days) of the three pools. "Pool 2" includes primarily those soft tissues, which equilibrate more slowly with whole blood and possibly parts of the skeleton where more active (and relatively rapid) exchanges occur with the central compartment (i.e., rapid relative to exchanges with "Pool 3"). As Pb is added to the system, BPb rises and it becomes an early indicator of Pb absorption in the body.   "Pool 2" represents less than 0.3 percent of the Pb-body burden, and relatively little of the Pb in this pool is returned to blood. "Pool 3" represents approximately 94–99 percent of the Pb-body burden and is assumed to include bone and other slowly exchanging tissues. Approximately 94 percent of the total body

burden of Pb is found in the bones of human adults. However, in children, bone Pb accounts for about 73 percent of the body burden (Barry et al., 1970). Concentrations of bone Pb-burden (mass) tend to increase with age, indicative of a relatively slow turnover of Pb in adult bone (Barry et al., 1970; Gross et al., 1975; Schroeder et al., 1968). The large pool of Pb in adults can be the source of BPb levels even long after external Pb exposure may have ended (Fleming et al., 1997; 1996; Smith et al., 1996).

From calcified tissue stores in the bones, Pb can be slowly released back into the soft tissues. This release depends mostly on bone turnover rates that are largely a function of the bone type. The compact bones have a slow turnover and the trabecular have a more rapid turnover (O´Flaherty et al., 1995). The release rate of Pb from bones varies with age and intensity of exposure (Brito et al., 2002). Continuous growth and constant bone remodeling for skeletal development (O´Flaherty et al., 1995) can be a source of Pb exposure. This change contributes to a state in which Pb that is stored in bone is released back into the blood stream described as "endogenous contamination" (Gulson et al. 1996A). Bone remodeling in pregnant women can also serve as a significant continuous source of BPb that is passed to the fetus during the formation of the fetal bone structures in the womb during gestation (Gulson et al, 1996b). Approximately 54–78 percent of the Pb which leaves the body each day in urine is assumed to come from the central compartment, while other excretion pathways (e.g., bile, hair, sweat, and nails) are assumed to originate from "Pool 2" (Rabinowitz, 1976 in EPA, 2001). Though this has been the most widely accepted model, it has been criticized on the grounds of differences of rates of Pb exchange in the different bones. Specifically, the development of a five-compartment Pb model (Bernard et al., 1977) was a result of an observation that the Pb exchange rate in trabecular (spongy) bones has a more rapid exchange Pb rate that cortical bone, and a wide

variation in concentration of Pb in various parts of the skeleton (Landrigan et al., 1985). This model, in addition to the Rabinowitz's model of blood and soft tissue, adds three separate compartments that include cortical bone, trabecular bone and remaining soft tissue.

Although the Rabinowitz model seems to be the least complicated of the Pb compartmental kinetics models, it is a basis for the more complex models that followed (*e.g.*, Leggett, 1993; O'Flaherty 1991). Differing from Rabinowitz's model, the Leggett (1993) model includes a larger number of tissue compartments (Figure # 2-4). Pb movement and deposition of in the body as exchanges between a various tissue compartments (and sub-compartments) and a central diffusible plasma compartment are simulated in this model. Pb bound in plasma proteins, brain, extra-vascular fluid, gastrointestinal tract, kidney, liver, lung, other soft tissues, red blood cells, and skeletal tissues (cortical and trabecular bone) are the main tissues represented in this model. In addition the model includes a number of excretory routes: feces, sweat, urine, and other routes (*e.g.*, hair, nails, and skin). According to EPA, the strength of the Leggett model is that it is an all-ages model that can simulate Pb accumulation in a variety of tissues over any selected age range for a wide range of Pb intake patterns, including intakes that vary in intensity over time. Furthermore, EPA states that the Leggett model works well over a wide variety of conditions as assessed by comparison between predicted and observed BPb concentrations and urinary or fecal excretion in adults and comparisons of predicted and observed post-mortem tissue Pb concentrations. The flexibility of the model makes it relatively easily to simulate complex Pb scenarios, including those that might represent baseline exposures (US EPA, 2001).

Another widely used model is the O'Flaherty model (Figure 2-5) which can simulate BPb concentrations for ages from birth through adulthood, for both short and long-term exposures. This model also simulates difference in Pb biokinetics in females and males. In addition,

according to EPA, the model can calculate tissue Pb accumulation in any compartment for any age range; simulate nonlinear kinetics of lead in blood; can be calibrated to achieve a reasonable fit to epidemiologic and experimental data and; and can generate output that includes graphics (US EPA, 2001). However, this model includes a number of significant limitations: relatively weak empirical support for some of the model components; not designed to simulate maternal biokinetics during pregnancy; the exposure module for adults is limited to age-specific intakes (US EPA, 2001). In their evaluation, EPA did not find any model to be a significant improvement over their Technical Review Workgroup for Lead (TRW) - Adult Lead Methodology (ALM) which is used to assess lead risks from the soil at nonresidential Superfund sites. The baseline blood lead concentration input parameter of the ALM represents the geometric mean blood lead concentration in women of child-bearing age and the geometric standard deviation (GSD) input parameter is a measure of the inter-individual variability in these concentrations (US EPA, 2001).

However they conclude that ALM, various components from the different models were determined to be refinements in adult lead modeling (US EPA, 2001). Furthermore, they state that some components could be integrated into a hybrid model, however, EPA's ALM should be retained as an interim methodology (US EPA, 2001)

Compartments and Pathways of Lead Exchange in the Leggett Model
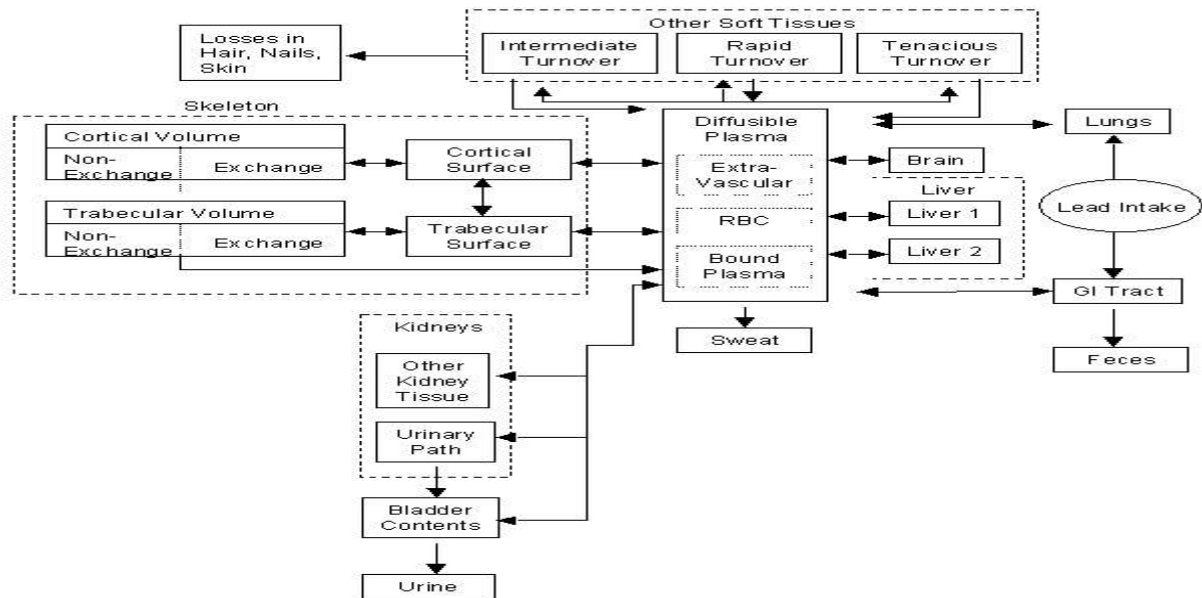
Source: Derived from Leggett 1993

Note: Transfer rates for exchanges between compartments are age-specific. See Leggett (1993) for the age-specific transfer rates used in the model.

Figure2-4: Schematic of the biokinetic model of lead metabolism proposed by Leggett (1993); Review of Adult Lead Models Evaluation of Models for Assessing Human Health Risks Associated with Lead Exposures at Non-Residential Areas of Superfund and Other Hazardous Waste Sites Final Draft: August 2001. Source: EPA, Adult Lead Risk Assessment Committee of the Technical Review Workgroup for Lead (TRW).
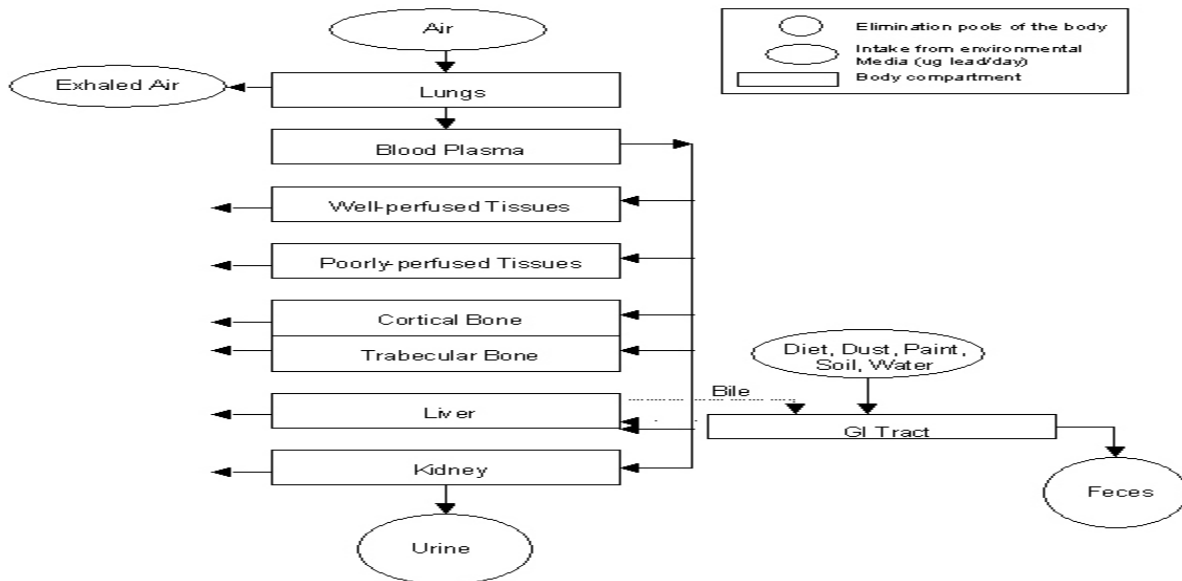
Figure 2-5: Compartments and pathways of lead exchange in the O'Flaherty model (1995) Review of Adult Lead Models Evaluation of Models for Assessing Human Health Risks Associated with Lead Exposures at Non-Residential Areas of Superfund and Other Hazardous Waste Sites Final Draft: August 2001. Source: EPA, Adult Lead Risk Assessment Committee of the Technical Review Workgroup for Lead (TRW).

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Pb toxicity may largely be explained by its interference with different enzyme systems: Pb inactivates enzymes by binding to SH-groups of its proteins or by displacing other essential metal ions (WHO, 2001). For this reason, many organs or organ systems are potential targets for Pb where a wide range of biological effects have been documented (WHO, 2001). There are two principal mechanisms of Pb toxicity: First, Pb complexes with important functional chemical groups including –COOH, –NH2 and –SH and so disrupts the function of enzymes and other biologically important molecules (WHO, 2001, Bradberry et al., 2007). Secondly, Pb substitutes for divalent ions, particularly calcium, with the potential for widespread chemical interactions both at cell membranes and within intracellular organelles (Goodwin, et al., 2001). The chemical similarity between Pb and calcium partly explains why these elements appear to be interchangeable in bone, and why more than 90 percent of the total Pb-body burden is stored in the skeleton. Among the most important enzymes disrupted by Pb are several that are involved in heme synthesis as shown in Figure 2-6 below.
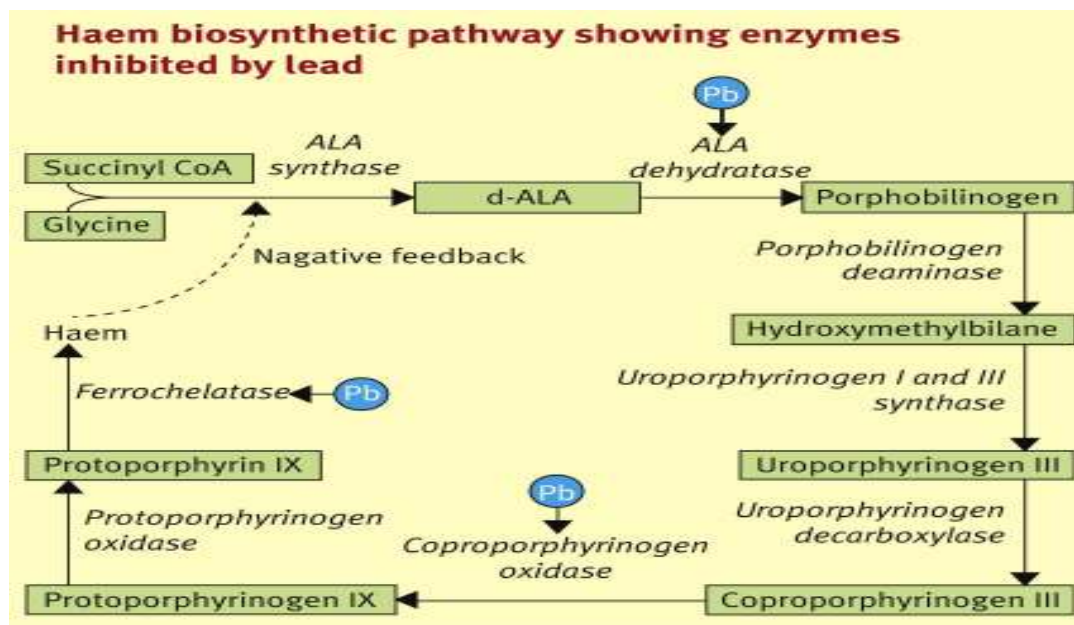
Figure 2-6: Heme biosynthetic pathway showing enzymes inhibited by lead. Source: Sally Bradberry, Allister Vale. Poisonous Substances-Lead. MEDICINE, 2007; 35:12: 627-628

Because heme is important for the function of erythropoiesis, inhibition of heme synthesis has a wide impact. δ-Aminolevulineate dehydratase (ALAD), the enzyme which catalyzes the formation of the heme precursor porphobilinogen, is highly sensitive to inhibition by Pb, leading to increased δ-aminolevulinic acid in the blood and urine (Warren et al., 1998). As described by Bradberry and Vale (Bradberry, et al., 2007), δ-aminolevulinic acid resembles γ-aminobutyric acid (GABA) and can stimulate GABA receptors in the nervous system; this is thought to be one of many mechanisms of Pb-induced neurotoxicity. In addition, increased concentrations of red cell zinc protoporphyrin (ZPP) occur due to inhibition of ferro-chelatase which normally incorporates iron into the heme precursor, protoporphyrin IX. When this process is blocked protoporphyrin chelates zinc in place of iron and ZPP accumulates (Bradberry, et al., 2007). Red cells containing ZPP are intensely fluorescent, and this has been used to diagnose Pb toxicity. Pb also inhibits erythrocyte pyrimidine-5′-nucleotidase resulting in the accumulation of pyrimidine nucleotides as "basophilic stippling" of immature erythrocytes, thereby contributing to increased red cell membrane instability and hence a shortened red cell lifespan. Pb poisoning thus results in anemia secondary to impaired heme synthesis and cell death-hemolysis (Bradberry et al., 2007). The US EPA concludes that scientific evidence presented to date clearly demonstrates deleterious Pb effects on erythrocyte cell morphology and function, as well as Pb uptake and alterations in certain enzymes involved in heme synthetic pathways (US EPA (2006b).

2.4 Measurement of Lead in the Body

BPb levels reflect an individual's current body burden, which is a function of recent and/or past exposure. Therefore, selection and measurement of appropriate biomarkers of Pb exposure is of critical importance. Whole BPb is the most widely and easily attained method of measurement of Pb in the body. This marker represents a recent Pb exposure because its half-life in the blood is approximately 30 days. However, this may also reflect the cumulative Pb exposure since the Pb in different compartments (bone, soft tissue, and blood) tends to be at equilibrium. As such, BPb has been the primary method used in order to determine the exposure to Pb and its risk assessment for any particular study population (i.e., environmental or the occupational setting). BPb concentration has remained the most widely used Pb exposure index in epidemiologic studies from 1990 to the present (US EPA, 2012). Obtaining a BPb sample is relatively noninvasive and quick. The laboratory techniques are well standardized and relatively inexpensive with wide access to quality controls, and existing regulation and medical decision-making are based on BPb levels (US EPA, 2012). It is generally accepted that if exogenous Pb exposure were the only determinant for BPb concentration, then a single BPb measurement would reflect very recent exposure (i.e., 30-90 day period prior to the analytical measurement). However, analytical measurements of BPb concentration represent a combination of recent exposure to external sources as well as the influence of internal sources, mainly Pb that has been stored in the bones. Analytically, BPb can be measured using one of the following methods: graphite furnace atomic absorption spectrometry (GFAAS), anodic stripping voltammeter (ASV), flame atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-

MS). The first two methods are generally considered to be the methods of choice for most laboratories (Flegal and Smith, 1995; EPA, 2006).

Exposure to Pb can also be measured indirectly through its effects on the hemopoietic system, mainly by interfering with a number of steps in heme synthesis (Piomelli et al., 1973). The excess accumulation of ZPP as a result of the inhibition of the enzyme ferrochelatase is most readily measured (Piomelli et al., 1973). The ZPP test, once widely used for primary screening, provides early indication of Pb poisoning and increase exponentially with Pb levels and is practical for quick screening of BPb levels (Piomelli et al., 1973). Ferrochelatase catalyzes the last step of heme synthesis, a process that occurs in the mitochondria of red blood cells; since circulating (mature) red blood cells do not contain mitochondria, ZPP reflects Pb exposure to immature red blood cells still in the bone marrow. Because the average life span of red blood cells is approximately 120 days, ZPP concentrations reflect average exposure to Pb over the last four months (Piomelli et al., 1973). Exposure to Pb also can be evaluated by measuring ZPP in blood samples where its been known to increase when the amount of lead in the blood is high, with a delay of a few weeks (Patrick et al., 2006; Kosnet et al., 2006). Thus, ZPP levels in conjunction with BPb levels can suggest the exposure period. In a case where the BPb levels are high but ZPP is still normal, this suggests a recent exposure (Kosnet et al., 2005 /2006)

The limitations for using ZPP as a biomarker include the facts that ZPP rises physiologically in the presence of iron deficiency and EP does not rise significantly in children until the BPb reaches 15-20 μg/dL (Piomelli et al., 1982). As a result of such a high threshold for detection, and the fact that EP levels also increase in iron deficiency, use of this method for detecting lead exposure has decreased (Grant et al., 2009).

Bone, where Pb is stored by replacing calcium of the hydroxy-apatite crystals of the bone mineral, is the main repository for chronic Pb stores in the body (Hu et al. 1998).   Pb is released into the blood stream and circulates throughout the body through a continual process of bone remodeling, synthesis, and reabsorption. A noninvasive *in vivo* X-ray fluorescence (XRF) method can be used in order to determine the Pb levels stored in the bones. However, as a result of a number of issues (such as operational cost, radiation exposure, and low specificity and sensitivity) this technique has remained a research tool rather that a screening method. Over the last decades, XRF bone-Pb measurements have become increasingly utilized in research.   K-shell X-ray fluorescence (K-XRF), a proxy for current whole-body Pb content, is used in measuring current or chronic Pb concentration in bone (Hu et al. 1991; Todd and Chettle 1994). Pb also accumulates in teeth. Tooth (dentine) Pb measures have also been used to assess the Pb-body burden.   One of the drawbacks of dentine measure is the fact that as deciduous teeth start shedding it is only possible to evaluate children over 6 years of age. The influence of age and/or sex have to be considered when assessing tooth Pb concentrations (Brown et al., 2002). Furthermore, variation of Pb concentrations from tooth to tooth, where it was observed that the concentrations of Pb in pairs of central and lateral incisors from children revealed a large difference for many individuals (Delves et al., 1982)

Many years ago, Pb measurement via a proxy biomarker in urine was favored for long-term biomonitoring of occupational Pb exposures. δ-aminolevulineate (ALA) is a substrate of an enzyme aminolevulinic acid dehydratase (ALAD) involved in heme synthesis is excreted and can be measured in urine. However, because of a number of limitations, this method is no longer widely used to assess the Pb body burden. For example the test requires a careful 24 hour urine collection (Wedeen et al., 1979; Osterloh et al., 1989).

2.5 Adverse Health Effects of Lead

Pb is a toxic metal that can affect virtually every system in the body. In general, children are more vulnerable to Pb exposure than adults. One of the reasons for this is the frequency of children's hand-to-mouth activity, as well as a higher rate of intestinal absorption and retention of Pb. At very high blood levels ($\geq$ 80 µg/dL of blood) Pb can cause convulsions, coma, and even death (EPA, 2012). Adverse health effects on the central nervous system, kidney, and blood cells are also possible at what once were considered as low levels. BPb levels as low as 5 ug/dL can impair mental and physical development. This represents the levels of the 97.5[th] percentile of NHANES distribution of BPb in children. Health effects of Pb include effects on heme biosynthesis, the nervous system, the kidneys, cardiovascular, hepatic, endocrinal and gastrointestinal systems (EPA, 2012; ATSDR, 2007; WHO, 2001). The most deleterious effects of Pb are on erythropoiesis, kidney function, and the central nervous system (ATSDR, 1993; Bellinger et al., 1995). In the last few decades, the effects of environmental Pb exposure have been examined in relation to human health. These evaluations involved populations with extremely high exposure levels (occupational studies), as well as those with relatively low level exposures (population studies discussed in the next chapter).

Numerous studies confirm that even slightly elevated BPb levels can be associated with learning and behavioral disorders-including delinquent conduct (Needleman et al., 1979; Dudeck et al., 1997). In 1979, Needleman et al. studied the effect of low-level Pb exposure on the psychological development and classroom performance of children and found that verbal and auditory processing, attention, and classroom behavior are most sensitive to Pb effects, even after controlling for confounding factors (Needleman et al., 1979). They found that the first and second grade children who had no symptoms of Pb poisoning, but who had elevated dentine Pb

levels, had lower scores in intelligence testing, speech and language processing, attention span, and overall classroom performance (Needleman et al., 1979).   Though this cross-sectional study had a number of limitations, many consider this study to be the one that opened the "flood gates" on this topic. Initially, an EPA panel reported a number of study limitations which included questionable design methods where school children were asked to submit their own shed teeth (Palca, 1991). Questions about whether they submitted their *own* teeth were also raised.   In addition, the study did not control for the confounding factor of the children's ages. When factoring in the age of children, other researchers noticed significantly different results. Also, the children who were "Pb poisoned" but without impaired intelligence, were excluded from analysis, and other results that did not support Needleman's conclusion were omitted (Palca 1991; Lewis, 1995). The EPA panel had initially decided that the study did "neither support nor refute the hypothesis that the low to moderate levels were associated with cognitive or behavioral impairment in children" (Palca, 1991). However, the same panel later reversed their conclusion and all of the charges of "scientific misconduct" were later refuted (Thomas, 1995). Additionally, in a follow-up to this study-11 years later, Needleman et al., found that the associations reported earlier between Pb and children's academic progress and cognitive functioning persisted into young adulthood. The persistent toxicity of Pb was seen to result in significant and serious diminished academic success; higher dentine Pb levels in children were later associated with a higher odds ratio for failure to graduate, lower class rank, increased absenteeism, lower scores on vocabulary and grammatical-reasoning tests, significantly slower finger-tapping speed, longer reaction times, poor hand-eye coordination and lower reading scores (Needleman, 1990).

The studies of the 1970's discussed above provided additional momentum for a number of long-term prospective studies that were designed to monitor Pb exposure and outcomes over time in the US and abroad. More specifically, the Port Pirie, Australia (McMichael et al., 1988), Dunedin, New Zealand (Silva et al., 1988), Cleveland, Ohio (Ernhart et al., 1989), Boston, Massachusetts (Bellinger et al., 1991), Cincinnati, Ohio (Dietrich et al., 1991), and Kosovo, Yugoslavia (Graziano et al., 1994) studies sought to elucidate long-term effects of Pb on children. Studies in Kosovo showed that prenatal and early childhood lead exposure was associated with poorer intellectual functioning at ages 2, 4, and 7 years (Factor-Litvak et al., 1999). In addition, a longitudinal study of this cohort reported a slight association between BPb and blood pressure (Factor-Litvak et al., 1996) and effects on the erythropoietin production (Factor-Litvak et al., 1998, Graziano et al., 2004).

As a result of these and other studies conducted in the last several decades, the Center for Disease Control and Prevention (US CDC) has been issuing guidance that identified levels of concern for BPb of young children. In the early 1970s, the acceptable value for Pb in blood was 40 µg/dL. It was lowered to 30 µg/dL in 1975; to 25 µg/dL in 1985; to 10 µg/dL in 1991 (CDC, 1991) and to 5 µg/dL in 2012. Data collected as part of the 2011 U.S. National Health and Examination Surveys (NHANES) showed the 97.5th percentile for BPb as 5.0 µg/dL for children 1−5 years of age, and as 5.20 µg/dL for adults 20 years of age and older [U.S. Centers for Disease Control and Prevention (CDC) 2011].

Although the BPb levels of U.S. populations have dropped markedly compared to 30 years ago, new concerns have been raised regarding possible adverse health effects in children at BPb levels < 10 µg/dL; perhaps there is no safe threshold, but rather a continuum of toxic effects (Canfield et al. 2003). In light of these concerns, the CDC Advisory Committee on

Childhood Lead Poisoning Prevention formed a working group to review the evidence for adverse health effects at BPb levels < 10 µg/dL in children. However, in a statement on the topic in 2005, the CDC noted that adverse effects of Pb on cognitive development, a key health endpoint of concern, extend to BPb concentrations of less than 10 µg/dL, and that there is no value that constitutes a threshold or no-effect level (CDC 2005b). Recently, the BPb levels have been reduced to 5 µg/dL (CDC, 2012) to reflect the lower 95th percentile of NHANES BPb levels in children from 1-5 years of age. Figure 2-7 and 2-8 below show the trend in BPb concentrations in U.S. children (1-5 years of age), and children 1-5 years of age and adults, respectively with geometric means and 95% confidence intervals as reported from the NHANES II, III and IV.
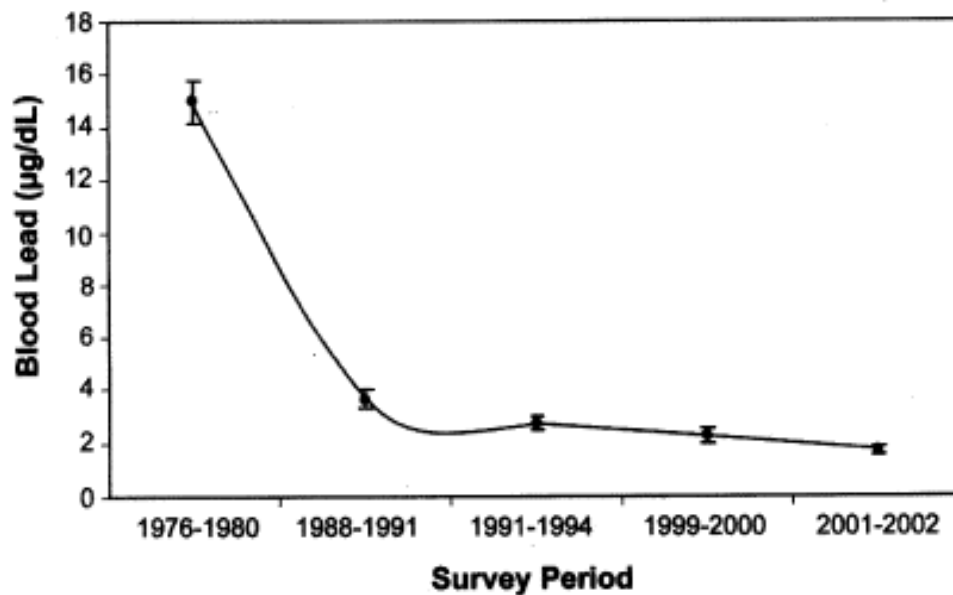
Figure 2-7: Blood lead concentrations in U.S. children, 1-5 years of age. Shown are geometric means and 95% confidence intervals as reported from the NHANES II (1976-1980) and NHANES III Phase 1 (1988-1991; Pirkle et al., 1994); NHANES III Phase 2 (1991-1994; Pirkle et al., 1998); and NHANES IV (1999-2000, 2001-2002; Centers for Disease Control and Prevention, 2005). Source: US EPA, 2006b
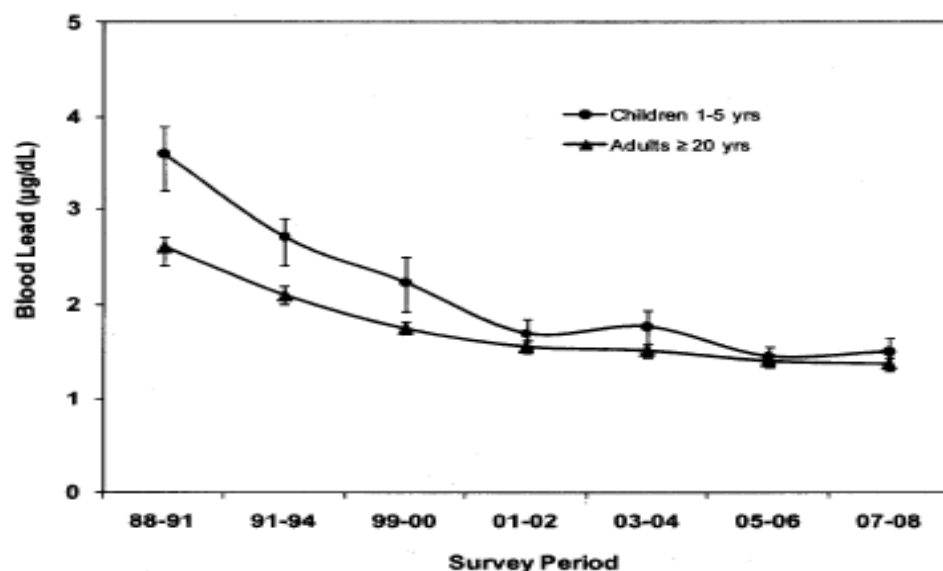
Figure 2-8: Shown are geometric means and 95% CIs based on data from NHANES III Phase 1 (Brody et al., 1994; Pirkle et al., 1994); NHANES III Phase 2 (Pirkle et al., 1998); and NHANES IV (CDC, 2011). Data for adults during the period 1988-1994 are for ages 20-49 years, and ≥ 20 years for the period 1999-2008. Source: US EPA, 2012

With regard to occupational exposure to Pb, the US Occupational Safety & Health Administration (OSHA) lead standard [29 Code of Federal Regulations 1926.62 and 1910.1025] established general industry standards for Pb in the late 1970s to prevent Pb exposure to adults in the workplace. Under the OSHA general industry Pb standard, which remains in effect to the current time, a worker requires removal from Pb exposure if a single BPb level exceeds 50 µg/dL, or if the average of the three most recent BPb measurements exceeds 50 µg/dL (provided the last is greater than 40 µg/dL). Nevertheless, studies conducted in recent decades, some of which are discussed in the next section, demonstrate that current Pb standards, be it in the occupational setting or environmental exposure, may offer inadequate protection against the adverse effects of Pb even at lower doses. A recent report by the National Research Council (2012) concluded that the OSHA Pb standards are not sufficiently protective.

2.6 Effects of Lead on Cardiovascular Disease

Exposure to Pb has been associated with an increased incidence of coronary heart disease, increased blood pressure, peripheral arterial disease, and stroke (Harlan et al., 1988; Pirckle et al., 1985; Navas-Ancien et al., 2007).   The association between BPb and blood pressure has been studied extensively, and there is a general consensus that supports an association between increasing BPb and blood pressure (Nawrot et al., 2002; Navas-Acien et al., 2007). A significant number of reviews and meta-analyses that included analyses of more than 30 studies and more than 50,000 participants have examined this relationship (Hertz-Picciotto and Croft 1993; Nawrot et al. 2002; Schwartz 1995; Sharp et al. 1987; Staessen et al. 1994, 1995; U.S. Environmental Protection Agency (U.S. EPA, 2006). An increase in systolic blood pressure that is associated with a doubling of BPb levels (e.g., from 5 to 10 µg/dL) was estimated to range from 0.6 to1.25 mmHg (Staessen et al. 1994). Another meta-analysis reported an average of 1.2 mmHg of sBP increase for every doubling of BPb concentration (Schwartz, et al., 1995). Even with significant reductions in environmental Pb exposure in the last 20-30 years, Pb exposure continues to pose a public health hazard.

The biological plausibility of this relationship has been documented in a number of animal studies. In rats, exposure to Pb for 4-5 months (leading to BPb concentration of 30-40 µg/dL) was reported to induce hypertension (Vander et al. 1988; Nowak, et al. 1993). Other more recent animal studies continued to provide evidence that long-term Pb exposure results in sustained arterial hypertension after a latency period. Systolic BP increased in rats after exposure to 90-10,000 ppm Pb (as Pb-acetate in drinking water) for various time periods that resulted in blood Pb levels between 19.3-240 µg/dL (Mohammad et al., 2010; Zhang et al., 2009; Grizzo and Cordellini, 2008). Although the effects of Pb appear to be mediated through multiple modes

of action, alteration of cellular ion status (including disruption of calcium homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) seems to be the major unifying mode of action underlying all subsequent modes of action (EPA-2012). The disruption of the biological functions that can interfere with tightly regulated processes such as cell signaling, intracellular ion homeostasis, ion transport, energy metabolism, and enzymatic function are the some of possible ways of Pb-induced cardiovascular toxicity.

There are a number of ways Pb can affect the blood pressure including, hormonal and blood pressure regulatory system dysfunction, vasomodulation, cellular alterations, and oxidative stress. One plausible way is through adverse effects on the kidney renin-angiotensin system (Vander et al. 1988). Alteration of the adrenergic system by Pb exposure, which can increase peripheral vascular resistance and arterial pressure, may be one cause of Pb-induced hypertension. Also, lead induced nephrotoxicity is believed to be a possible precursor to increased blood pressure in humans (Cowley et al. 1996, Corrick et al. 1999). In addition, Pb may also act on many sites of the cardiovascular system that include the excitability and contractility of the heart, altering the smooth vascular tissue, or impact on the part of the central nervous system that regulates the blood pressure (Kopp et al. 1988). However, it is possible that other interrelated factors may contribute to the raised blood pressure. Hypertension is a disease with a multi-factorial etiology, and therefore it is likely that risk factors will differ among races and between genders. Their relative roles may differ between individuals, because of the biological inter-action of nutritional and behavioral lifestyle, genetic makeup, and environmental exposure to various other agents.

In addition, Pb has been associated with other cardiovascular function abnormalities that can be tied to elevated blood pressure such as left ventricular hypertrophy and alterations in cardiac rhythm (Navas-Ancien et al., 2007). Also, elevated Pb exposure induces oxidative stress and inflammatory/biochemical markers which may be used to detect impairment in the body function in Pb-exposed workers (Khan et al., 2008). Indeed, Pb-induced oxidative stress via generation of reactive oxygen species (ROS) has been thought to be a primary contributory factor in the pathogenesis of its adverse health effects (Vaziri & Khan et al., 2007). In turn, ROS cause damage at the cellular level, i.e., on cellular function and damage to DNA, proteins, and lipids (Ahamed et al., 2002). A number of studies have demonstrated a role for oxidative stress in the pathogenesis of Pb-induced hypertension, mediated by the inactivation of nitric oxide (•NO) and down-regulation of soluble guanylate cyclase (sGC) (Dursun et al., 2005; Vaziri et al., 1997). The reduction of the vasodilator •NO can lead to increased vasoconstriction and in turn increased blood pressure. In Pb-exposed rats, Pb accumulates in the vasculature even after the exposure has ended (Malvezzi et al., 2001). It is believed that this is a contributing factor to the stiffness of the arteries and therefore believed to be responsible for increased systolic blood pressure and widening pulse pressure (Pearlstein et al., 2007). However, the same stiffness of the large arteries may be responsible for decreased diastolic blood pressure and widening of the pulse pressure (Mitchell et al., 2004; Lakatta, et al., 2003). The difference between the sBP and the dBP, known as pulse pressure, is an indicator of, as well as a risk factor for, cardiovascular disease (Safar et al., 2003). This implicates vascular accumulation of Pb in the pathogenesis of vascular stiffening and arterial aging. Furthermore, these mechanisms are consistent with the findings of aortic Pb deposition in humans and increased vascular oxidative stress in the Pb-

exposed animals', perhaps providing mechanistic insight into the observed association of low-level Pb exposure with cardiovascular mortality (Pearlstein et al., 2007).

It has also been reported that Pb may be an important factor in stimulation and proliferation of vascular smooth muscle cells (Fujivara et al., 1995). Recent studies indicate that the concentrations of the circulating endothelial soluble intercellular adhesion molecule (sICAM-1) and soluble vascular cell adhesion molecule (sVCAM-1) appear to have predictive value for the identification of early atherosclerotic lesions and future cardiovascular disease (CVD) (Krauss et al., 2002; Blankenberg et al., 2001; Ridker et al., 2000; Hwang et al., 1997). These adhesion molecules play a crucial role in the immune system response by promoting cell–cell and cell–stroma interactions and leukocyte migration (Carlos & Harlan, 1994). The process of adhesion of the leukocytes to the endothelial cells and subsequent trans-endothelial migration is an important step in the atherosclerosis (Ross, 1993). The trans-endothelial process allows for leukocytes to migrate to sites of inflammation, which in turn can play a role in the initiation of diseases such as cancers and arthritis (Polverini, 1997). While studies have shown that age is the most powerful independent predictor of the increasing levels of sICAM-1 and sVCAM-1, the effects of Pb may also play a significant role in this process (Morisaki et al., 1997). Both sICAM-1 and sVCAM-1 are considered proinflammatory factors and are expressed during vascular inflammation and endothelial dysfunction and can be detected in the circulation. Elevated sICAM-1 levels have been associated with CVD risk factors such as hypertension, smoking and frequent alcohol consumption (Blann et al., 1997; Rohde et al., 1999). As a possible proinflammatory factor, sICAM-1 were found to be related to increasing systolic blood pressure (Chae et al., 2001). Angiotensin II, which is a potent vasoconstrictor, stimulates the

sICAM-1 expression and as such, it may play a role in the increase in blood pressure (Mervaala et al., 1999).

There are a number of epidemiological studies that have reported on the positive association of these adhesion molecules and risk of cardiovascular disease (Ridker et al., 2000; Hwang et al., 1997). Studies have also shown a positive relationship between chronic arsenic exposure and sICAM-1 and sVCAM-1 (Chen et al., 2007). However to our knowledge, there have not been any epidemiologic studies that have examined the associations between Pb exposure and its effect on the circulation levels of sICAM-1 and sVCAM-1. This dissertation project therefore also includes measurements of circulating levels of sICAM-1 and sVCAM-1 in study participants.

It is important to point out that the blood pressure measurements are inherently variable and can vary significantly in the same individual. The measurements can be influenced by extrinsic factors such as the technique utilized for the measurement or by the instruments used. Since blood pressure measurements can vary significantly between readings, taking the average of the continuous readings may reduce the probability that error may be entered into the outcome. Noticeably, the errors in measurement tend to be additive, and the total error will most likely be symmetrically distributed about some true and unbiased result, with larger studies providing a greater a chance of detecting the true effect (EPA, 2006). In addition, because of a number of blood pressure determinants, confounding is a possibility that needs to be addressed. More specifically, a well designed study model must address potential confounders such as demographic (age, gender etc.), body mass index, smoking etc.) and environmental or genetic contributors can affect the outcome. In summary, support for a Pb and blood pressure

relationship comes from clinical, epidemiological studies in workers exposed to Pb, as well as in general population groups exposed to environmental Pb.

2.7 General Population Studies

Association between BPb and blood pressure have been explored in a significant number of cross-sectional population studies during the last two-three decades (Table 2-2). Among them, the three large population-based studies, the British Regional Heart Study (BRHS), the National Health and Nutrition Examination Survey (NHANES II and III), and a smaller Welsh Heart Programme comprise the main body of scientific research that observed associations between BPb levels and increased blood pressure.   In addition to employing well established standards and highly trained research staff, these studies also draw from relatively large sample sizes; therefore they can likely minimize errors and inherent disadvantages that can result from a lack of extremely precise exposure measurements/assessments.   The first two studies, BRHS and NHANES II and III studies showed weak correlations between BPb levels and increases in blood pressure or hypertension, but the Welsh study did not show a significant correlation (REIS-1994).

Table 2-2: Selected Population Studies of Pb and Blood Pressure Associations

Researcher/s	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Pocock et al, 1984	Clinical survey of 7,735 men (40-49 yrs old) BRHS	Renal function, BP	8.2 and 29.1 ug/dL	Mean increase 1.45 mmHg sBP for doubling of BPb
Pirkle et al, 1985 Harlan et al,1988 Landis & Flegal, 1988	Cross-sectional survey, NHNES II 12 to 74 years	Blood pressure	Mean BPb 13.1 ug/dL	sBP and dBP stat. significant with BPb in men. Raising the BPb from 10-30 μg/dL, the sBP and dBP increased by 9.26 and 4.34 mmHg
Elwood et al, 1988	Cross-sectional study, Wales towns M- 49 to 65; W 18-64 years of age	Blood pressure	Mean BPb= Men = 13.1 μg/dL Women = 9.6 μg/dL	No effect; but if real effect, 0.7 mmHg would increase for every mean of 10 μg/dL
Schwartz et al, 1988, 1991	Cross-sectional survey of NHNES II for White males 40 to 59 years	Blood pressure (Diastolic only)	Mean BPb 13.1 ug/dL	Increase of 2.03 mm Hg diastolic BP (95% CI: 0.67, 3.39) for males and a 1.14 mm Hg (95% CI: 0.13, 2.08) for females for every doubling of BPb.
Pocock et al, 1988	Compared the results of the British, NHANES II, and the Welsh study	Blood pressure	8.2-29.1 ug/dL ; Geo. mean 12.8 ug/dL; men = 12.4 μg/dL women = 9.6 g/dL	Increase of about 1 mm Hg for every doubling of blood lead concentration.
Hu et al, 1996	Case-control Normative Aging Study-30 year longitudinal study	Blood pressure (bone lead)	1-28 μg/dl (mean blood Pb 6.3)	Increasing bone Pb from low to highest quintile, associated with a 1.5 odds ratio for hypertension
Scinicariello et al, 2010	NHANES II dataset from all adults 17 years of age and up	ALAD2 Gene/Hypertension interaction	Mean BPb 13.1 ug/dL	Two-fold BPb conc. Increases sBP:1.91mmHg BM; 0.62mmHg WM; 0.65 mmHg WW 0.92 mmHg Mex–Ame men
Scinicariello et al, 2011	Cross-Sectional -BPb and BP study of NHANES 1999–2006	sBP and dBP; pulse pressure hypertension status	Mean blood Pb 1.75 μg/dL	BPb levels were significantly correlated with higher systolic BP among black men and women, but not white or Mexican–American participants
Den Hond et al, 2002	NHANES III (1988-1994) dataset from all adults 20 years of age and up.	Blood pressure	Mean BPb 2.76 ug/dL	sBP=0.9 mm Hg males, 1.02 mm Hg in females for every doubling on BPb
Vupputuri et al., 2003	NHANES III (1988-1994) dataset from all adults >20 years	Blood pressure	< 0.8 to > 20.0 μg/dL (mean BPb 2.3 (M); 4.2 μg/dL (W)	sBP=0.25 mm Hg males, 0.47 mm Hg in females for every 1 μg/dL increase in BPb
Nash et al, 2003	NHANES III (1988-1994) dataset from 40 to 59 year olds	Blood pressure	0.5 to 31.1 μg/dL (mean 2.9 μg/dL)	Only dBP=0.32 mm Hg for every 1 μg/dl increase in BPb
US EPA, 2006, 2012	Review of published data	Blood pressure	1 > 40 μg /dL	2006= ~ 1mm Hg and ~0.6 mm Hg for every doubling of BPb. Bone Pb=Inc OR for Hypertension, and ~0.75 mm Hg for every 10 μg/g inc. in bone Pb

In their BRHS study, Pocock et al (1984) conducted a clinical survey of blood pressure, indicators of renal function, and BPb concentrations in 7,735 men (ranging from 40-49 years old) from 24 British towns. They concluded that increased hypertension (systolic pressure greater than 160 mm Hg, diastolic pressure greater than 100 mmHg) was suggested at BPb concentrations > 37 μg/dL. After adjusting for relevant confounding variables, including town of residence and alcohol consumption, a weak but statistically significant positive association between BPb and both systolic and diastolic blood pressure was reported. These cross-sectional data indicate that an estimated mean increase of 1.45 mmHg in systolic blood pressure occurs for every doubling of BPb concentration (95% CI = 0.47, 2.43 mmHg) (Pocock et al., 1984). In comparing the results of the BRHS, NHANES II, and the Welsh study, Pocock concluded that blood pressure increases roughly 1 mmHg for every doubling of BPb concentration.

The NHANES II (1976–1980) study was conducted on 9,933 persons representative of the general non-institutionalized U.S. population at 6 months to 74 years of age; 2,372 were 6 months to 5 years old, 1,720 were 7-17 years old, and 5,841 were 18-74 years old (Harlan et al., 1985). The study observed significant correlations between BPb and blood pressure for each race-gender group, and BPb levels were significantly higher in groups with high diastolic blood pressure (> 90 mmHg) (Harlan et al., 1985). After adjusting for age, race, and body mass index, BPb levels were significantly related to systolic and diastolic pressures in males but not in females. Pirkle et al., (1985) re-examined the relationship between blood pressure and BPb levels in the NHANES II for white males aged 40–59 years (Pirkle et al., 1985). After adjustment for age, body mass index, and nutritional factors, the relationship of systolic and diastolic blood pressures to BPb levels was statistically significant ($p < 0.01$) with no evidence of

a threshold BPb level for this relationship. Raising the BPb from 10-30 µg/dL, the systolic and diastolic blood pressures increased by 9.26 and 4.34 mmHg respectively. The correlation was significant for BPb concentrations ranging from 7-34 µg/dL. These findings along with those from other studies suggested a relationship of BPb and blood pressure at levels commonly observed in the general population.

A study by Landis and Flegal (1988) tested the statistical significance of the association of diastolic blood pressure and BPb levels among the males ages 12-74 that were part of the NHANES II survey. In addition, their study sought to address some of the possible restrictions in previously reported studies (Harlan et al., 1985; Pirkle et al., 1985). Essentially, they used a complicated statistical procedure to evaluate the linear regression relationship between blood pressure and BPb levels, averaging across the sampling sites, age, and body mass subgroups. Thus, this method not only adjusted for the two primary covariates in characterizing the variation in blood pressure, but also adjusted for any measurable trends indirectly through stratification on the 64 sampling sites (Landis and Flegal, 1988). After adjusting for geographical location, age, and body mass, which resulted in 478 stratifications, diastolic pressure remained positively associated with BPb levels in white males aged 12-74.

A population-based study of two small groups of men and women in Wales evaluated the relationship between blood pressure and BPb. In contrast to the studies referenced above, the Welsh study did not observe a relationship between BPb and blood pressure. One of the study populations consisted of 1,137 men aged 49 to 65 years, the other of 865 men and 856 women aged 18 to 64 years. Neither population had any known significant exposure to Pb, and the values at the 95th percentile of BPb levels ranged from 6-26 and 5-18 µg/dL in the men and women respectively (Elwood et al., 1988). While this study did not show a significant

correlation between BPb and blood pressure, regression coefficients suggested that if there were a real effect, then the mean difference in blood pressure per 10 μg/dL difference in BPb is likely to be 0.7 mm Hg in both systolic and diastolic pressures (Elwood et al., 1988).

An association between BPb and systolic and diastolic blood pressure was also observed in NHANES II data among white males between 40 and 59 years old (Schwartz et al., 1988). They reported a robust relationship between low-level Pb exposure and blood pressure, and the relationship was significant regardless of whether they only examined a particular age group (with minimal age confounding) or whether they examined all adult men (Schwartz et al., 1988). Furthermore, they conducted regression analyses of electrocardiograms and blood pressure to evaluate the association of Pb and both outcomes in male and female participants aged 20 years and older. They concluded that BPb is a significant predictor of dBP and left ventricular hypertrophy, even after controlling for race, age, and body mass (Schwartz et al., 1991).

A dataset form NHANES III (1988-1994) from all adults 20 years of age and up was examined to evaluate the association between BPb and systolic and diastolic blood pressure (Den Hond et al., 2002). Utilizing multiple regression analyses for each blood pressure measurement, they stratified four models for each blood pressure measurement according to gender and race. The mean BPb levels were 3.6 μg/Dl for white males (n = 4,685), 2.1 μg/dL for white females (n = 5,138), 4.2 μg/dL for black males (n = 1,761), and 2.3 μg/dL for black females (n = 2,197). BPb ranged from < 0.8 to > 20.0 μg/dL. A significant association between BPb and systolic blood pressure was reported only in blacks. For each doubling in BPb, there was an associated sBP increase of 0.90 mmHg (95% CI: 0.04, 1.8) and 1.20 mmHg (95% CI: 0.4, 2.0) increase in males and females respectively (Den Hond et al., 2003). Though the study suggested differences between blacks and whites in response to BPb, no statistical tests were performed of differences

between BPb coefficients based on race (US EPA, 2006). In addition, the black-white effect differences associated with BPb may have been due to possible confounding in some or all of the models (US EPA, 2006).

Another analysis of the NHANES III dataset examined the differences in the BPb effect in terms of race and gender (Vupputuri et al., 2003). This study included 5,360 white men (mean blood Pb 4.4 µg/dl); 2,104 black men (mean blood Pb 5.4 µg/dl); 5,188 white women (mean blood Pb 3.0 µg/dl), and 2,300 black women (mean blood Pb 3.4 µg/dL). Results showed that only black men and women had significant linear BPb effects in adjusted sBP of 0.25 mmHg [95% CI: 0.06, 0.44] for black men and 0.47 mm Hg [95% CI: 0.14, 0.80] for black women with each 1 µg/dL increase in blood Pb). The impact on dBP was reported at 0.19 mmHg [95% CI: 0.02, 0.36] for black men and 0.32 mmHg [95% CI: 0.11, 0.54] for black women (Vupputuri et al., 2003).

Finally, in their work on NHANES III, Nash et al (2003) reviewed data from a sample of 40-59 year old women. They examined the relationship between BPb and both blood pressure (n = 1,786) and hypertension (n = 2,165) over a blood-Pb range of 0.5 to 31.1 µg/dl (mean 2.9 µg/dL) (Nash et al., 2003). Only sBP was significantly associated with BPb, where for each 1 µg/dL increase in BPb, there was a 0.32 mmHg (95% CI: 0.01, 0.63) increase in blood pressure.

The regression coefficients of studies using NHANES II data were generally higher than those reported by studies with current NHANES III. Also, the BPb mean levels in NHANES II were reportedly higher than the BPb in subsequent NHANES III surveys. The seemingly inconsistent results between the different studies are probably due to differences in covariates selected as risk factors and mean Pb concentrations (Scinicariello et al., 2011). The NHANES studies have several important strengths. Because the NHANES surveyed a large probability

sample of the general population, the findings can be generalized to U.S. adults. The large

sample size made it possible to detect small but important associations between BPb and blood

pressure. A limitation of these studies is that they are a cross-sectional study design, and

therefore limit the inferences that can be made. Another limitation is that although BPb reflects

both recent exogenous exposure and endogenous redistribution of Pb stored in bone, blood-Pb

concentration may underestimate the internal dose of Pb.

2.7.1 Bone Lead and Cardiovascular Disease

Approximately 95 percent of the total body burden of Pb is present in the skeleton and

measurement of bone Pb levels can provide a more valuable measure of internal dose (Hu et al.,

2007). Bone-Pb has been shown to be a better biomarker of Pb dose than BPb in term of

predicting hypertension in adults (Hu et al., 1996). Table 2-3 shows a number of studies that

compared associations of BPb and bone-Pb with blood pressure and hypertension.

Table 2-3: Selected Population studies that compared associations of <u>bone</u> lead with blood pressure and hypertension

Researcher/s	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Hu et al, 1996	590 participants of the Normative Aging Study	HTN	BPb 6.3 (4.1) ug/dL Tib-Pb 21.6 (12.1) ug/g	Tibia OR = 1.019 (95% CI: 1.004, 1.035) for HTN; BPb or patella-Pb not associated with hypertension.
Korrik et al, 1999	Study of 284 middle-aged women	HTN	BPb 13.3 (9.0) ug/dL Patella-Pb 17.3 (11.1) ug/g	Patella lead odds ratio for HTN = 1.03 (95% CI: 1.00, 1.05)
Schwartz et al, 2000	Cross-sectional analysis of 504 current/former chem. manufacturing in the eastern US	Blood pressure	Mean BPb 4.6 ug/dL Mean Tibial lead 14.7 µg/g	1 ug/dl increase in BPb-a 0.50 mmHg increase in sBP and 0.31 mmHg increase in dBP; BPb associated with HTN among workers <58 y/old; Tib-Pb not associated w/ sBP, dBP HYP
Cheng et al, 2001	590 participants of the Normative Aging Study	Blood pressure and hypertension	BPb 6.3 (4.1) µg /dL Tib-Pb 21.6 (12.1) µg/g	1 SD (12.7 ug/g) increase in Tib-Pb associated with a 1.37 mmHg increase in sBP; patella-Pb not associated with sBP; no associations reported for dBP.

Researcher/s	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Lee et al, 2001	Cross-sectional study of 798 Korean lead workers and 135 controls.	Influence of Vitamin D receptor (VDR) and ALAD on BP and HYP	Mean BPb 31.7-34.8 μg/dL Mean Tibia lead 37.5-38.1 μg/g	Tibia OR=1.005 (95% CI*: 1.000, 1.001) for hypertension; BPb and Tib-Pb not associated with sBP or dBP
Rothenberg et al, 2002	Study of 667 pregnant women	Blood pressure	BPb 2.3 (4.3) ug/dL Tib-Pb 8.0 (11.4) μg/g)	During 3rd trimester: a 10 ug/g increase in calcaneus-Pb associated with an odds ratio of 1.86 (95% CI: 1.04, 3.32) for HTN and a 0.7 mmHg (95% CI: 0.04, 1.36) dBP increase and 0.5 mmHg (95% CI: 0.01, 1.08) dBP increase; postpartum: BPb not associated with increased sBP/dBP.
Glen et al, 2003	496 current and former employees of a chemical-manufacturing in the eastern US	Blood pressure	BPb 4.6 (2.6) μg/dL Tib-Pb 14.7 (9.4) μg/g	SBP increased at an average annual rate of 0.64 mmHg and 0.73 mmHg for each 1 SD increase in BPb and Tibia-Pb, respectively
Martin et al, 2006	Community-based cohort of 964 men/ women:50–70 years old in Baltimore, MD	Blood pressure and Hypertension	Tib-Pb in blacks: 21.5 (12.6) μg/gr Tib-Pb in whites: 16.7 (11.9) μg/gr	1 log/dl increase in BPb was associated with a 0.99 mmHg increase in sBP and a 0.51mmHg increase in dBP. Tibia lead was not associated with sBP or dBP.

In a case-control study of participants in the Veterans Administration (now Department of Veterans Affairs) Normative Aging Study, a 30-year longitudinal study of men, Hu et al (1996) tested their hypothesis that long-term Pb accumulation of Pb in bone was associated with increased odds of developing hypertension, defined as a repeatedly elevated blood pressure exceeding 140 over 90 mmHg (Hu et al., 1996). Their study included relatively low BPb levels, ranging from < 1 to 28 μg/dL, with a mean (SD) 6.3 μg/dL. In comparison to non-hypertensives, mean levels of BPb and both tibia and patella bone Pb levels were significantly higher in hypertensive participants. Their findings suggest that long-term Pb accumulation, as reflected by levels of Pb in bone, may be an independent risk factor for developing hypertension in men in the general population. Furthermore, their study revealed that recent circulating dose, as estimated by BPb, was associated with systolic and diastolic blood pressure, while cumulative Pb

dose, as estimated by tibia Pb, was associated with clinically evaluated hypertension status. The contrasting associations seem to suggest a short latency, acute effect of BPb on blood pressure as well as a longer latency, chronic effect resulting in an increased risk of hypertension (Hu et al., 1996).

Others have also studied the associations between BPb, tibia Pb and cardiovascular outcomes. In several studies, BPb, but not tibia Pb, was associated with systolic and diastolic blood pressure, and this association was independent of tibia Pb; associations were larger with systolic, rather than diastolic blood pressure (Martin et al., 2006; Navrot et al., 2002; Hertz-Picotto et al., 1993). In contrast, tibia Pb was associated with an increased odds ratio for hypertension, but BPb was not, and its inclusion in the models did not alter tibia Pb associations (Martin et al., 2006). Similarly, in a cross-sectional analysis of 2001–2002 data from a community-based cohort of 964 men and women aged 50–70 years in Baltimore, Maryland, Martin et al, (2006) evaluated associations of BPb and tibia lead with systolic and diastolic blood pressure and hypertension while adjusting for a large set of potential confounding variables. They found that BPb was a strong and consistent predictor of both systolic and diastolic blood pressure in models adjusted and not adjusted for race/ethnicity and socioeconomic status (Martin et al., 2006). The magnitude of the associations was similar to that reported in other comparable studies.

Among published studies that looked at associations between bone PB and blood pressure, trabecular bone Pb has been most consistently associated with blood pressure outcomes (Korrik et al., 1999; Cheng et al., 2001; Rothenberg et al., 2002). In addition, trabecular bone Pb was associated with hypertension in a study of older men (Cheng et al., 2001) and women in the Nurses' Health Study (Korrik et al., 1999). This association has been also seen in younger

pregnant women (Rothenberg et al., 2002) in whom the association of calcaneus Pb was present only in the third trimester. However, BPb, tibia, and calcaneus Pb were not associated with hypertension after the end of pregnancy (Rothenberg et al., 2002). In the US EPA's review of 32 studies, over a range of bone Pb concentrations (<1.0 to 96 µg/g), every 10 µg/g increase in bone Pb was associated with an increased odds ratios (OR) for hypertension, with OR ranging from 1.28 and 1.86. Overall, the studies observed an average increase in systolic BP of ~0.75 mmHg for every 10 µg/g increase in bone Pb concentration over a range of <1 to 52 µg/g (US EPA, 2012).

Genetic differences in the enzyme ALAD appear to influence Pb toxicity, however, there are a limited number of studies that evaluated this association. Interactions between blood Pb and ALAD, which varied by race/ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American) were reported in one study (Scinicariello et al., 2010). In that study, BPb was associated with systolic blood pressure in non-Hispanic whites and with hypertension and systolic and diastolic blood pressure in non-Hispanic blacks. Non-Hispanic white ALAD2 carriers in the highest BPb quartile (3.8–52.9 µg/dL) had a significantly higher adjusted prevalence odds ratio for hypertension compared with ALAD1 homozygous individuals. The study concluded that BPb may be an important risk factor for hypertension and increased systolic and diastolic BP and that these associations may be modified by ALAD genotype (Scinicariello et al., 2010). A few other studies that evaluated the genetic differences in the enzyme ALAD and potential influence on Pb toxicity are related to occupational studies, and are discussed in chapter 1.7.3 on page 41.

2.7.2 Blood Lead and Blood Pressure in Children

There are only a limited number of published studies examined associations between Pb exposure and blood pressure in children. In one of the early studies, (Selbst et al., 1993) no association was observed between increased BPb levels and BP in a cohort of 149 children in Philadelphia (ages 1-10 years old). Another study of 121 children (mean age of 9.5 years old) enrolled in the Oswego Children's Study in NY (Gump et., 2005) found that an increasing *cord* BPb level was associated with significantly higher baseline sBP and marginally higher baseline dBP at birth. However, the same study did not find an association between blood pressure and *postnatal* BPb levels (measures at 2.5 years of age). In a large clinical study of 780 children at 12-33 months of age in several Northeastern US states, (BPb concentrations ranging from 20-44 ug/dL) researchers found no association between BPb and blood pressure (Chen et al., 2006). More recently, Zhang et al., (2012) examined children in Mexico City born from 1994 to 2003. The geometric mean cord and concurrent blood Pb levels of the children in the Mexico City cohort were 4.67 and 2.56 µg/dL. They found that *maternal* bone-Pb (tibia-Pb) was associated with increase in sBP and dBP only in girls. No associations were found between cord and *postnatal* (early childhood) BPb levels and blood pressure.

A study conducted by Factor-Litvak et al., (1996), examined associations between BPb concentration (BPb) and blood pressure in 282 children age 5.5 years, residing in exposed and unexposed towns in Kosovo. Mean BPb in the exposed town was 37.3 µg/dl (SD=12.0 µg/dL) and in the unexposed town was 8.7 µg/dl (SD=2.8 µg/dL). After adjustment, a 10 µg/dl increase in concurrent BPb was associated with a 0.5 (95% CL=-0.2, 1.3) mmHg increase in systolic and a 0.4 (95%, CL=-0.1, 0.9) mmHg increase in diastolic blood pressure. These associations are

most consistent with a very small association between BPb and blood pressure in children (Factor-Litvak et al., 1996).

Summarizing research on this topic, we can conclude that there is a small but consistent relationship between BPb and systolic/diastolic blood pressure across a wide group of populations. The data presented in these studies were comprised of populations that ranged significantly in age and exposure assessments, possibly confounding the early signs of the blood pressure at much younger age. We intend to deal with these specific inconsistencies by exploring the relationship between BPb and young men/women (25-26 years of age), with extremely well documented BPb histories during the first 12 years of their lives.

2.7.3 Occupational Studies of Blood Lead and Blood Pressure

Studies of occupationally Pb-exposed workers have also analyzed the relationship between BPb and BP parameters as it relates to their exposure measurements (Table 2-4). Occupational studies, which tend to rely on retrospective information and exposure assessment, can introduce a significant degree of bias. The bias in these studies can be introduced by misclassification of various components of the data collection and interpretation. However, whether it is the smaller size of the studies, poor statistical power, lack of adjusting for important covariates, "healthy worker effect" or some other factors, these studies can potentially hide the real effects.

Table 2-4: Selected Occupational Studies of Pb and Blood Pressure Associations

Researcher/s	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Kirby and Gyntelberg, et al, 1985	Case-control study of exposes and non-exposed lead smelter workers	Blood pressure	51 µg/dL exposed; 11 µg/dL for controls	Higher dBP values in both the supine and sitting positions, 4 (p < 0.04) and 5 (p < 0.05) mmHg, respectively in Pb workers.
deKort et al, 1986	Survey of 53 exposed and 52 non exposed – in Netherlands	Blood pressure	47.4 µg/dL compared with 8.1 µg/dL for controls	1.8 mmHg for sBP and 1.0 mmHg for dBP increase per 10 µg /dl increase in BPb level when a linear relation is assumed.
Weiss et al, 1988	Longitudinal study of 89 Boston policemen	Blood pressure	> 30 µg/dL	Association between high BPb and high sBP with value of 5.8 (C. I. 90%, 1.5-11.5 mm Hg) was reported.
Maheswaran et al, 1993	Cross-sectional study in 1981 on 809 male workers in lead and batteries factory, Birmingham, UK	Blood pressure	Mean BPb 31.6 µg/dL	sBP *(F= 3.3, p < 0.05)* with increasing blood lead levels, from 129 mmHg (95 percent CI 123.5-130.5) in to 132 mmHg in the higher BPb categories
Wu, et al, 1996	Cross-sectional study of 112 male and 110 female workers at two lead battery factories e 1992 study In Taiwan	Blood pressure	Average blood lead level was 56.9 ± 25.5 µg/dL	Short-term lead exposure or BPb level, was not related to blood pressure change among workers who had been exposed at work to occupational lead
Schwartz et al, 2001	Cross-sectional analysis of 504 current and former employees of a chemical-manufacturing facility in the eastern US	Blood pressure	Mean BPb 4.6 Mean Tibial lead 14.7 µg/dL	1 ug/dl increase in BPb-a 0.50 mmHg increase in sBP and 0.31 mmHg increase in dBP; BPb associated with HTN among workers <58 y/old; Tib-Pb not associated w/ sBP, dBP HYP
Lee et al, 2001	Cross-sectional study of 798 Korean lead workers	Vitamin D receptor VDR/ALAD & BP/ HYP	Mean BPb 31.7-34.8 µg/dL Mean Tibia lead 37.5-38.1 µg/dL	Tibia OR=1.005 (95% CI*: 1.000, 1.001) for hypertension; BPb and Tib-Pb not associated with sBP or dBP.
Glenn et al, 2003	Cross-sectional analysis of 496 current and former employees of a chemical-manufacturing facility in the eastern US	Blood pressure	Mean BPb 4.6 Tibial lead 14.7 µg/dL	sBP increased by 0.64 , 0.73 and 0.61 mmHg for any SD increase BPb baseline, Tib Pb at year three, or peak past Tib Pb lead, respectively
Weaver et al, 2008	Cross-sectional analysis of 652 current and former Pb workers Korean in a longitudinal study of the adverse health effects of inorganic Pb exposure	Blood pressure	Mean BPb 30.9 (µg/dL) Mean Patella Pb 75.1 µg Pb/g bone mineral	Positive association between BPb and sBP with β-coefficient, SEβ, and P-value respectively (0.1007, 0.0404, 0.01) Patella Pb does not appear to be a significant risk factor

In their investigation of the coronary risk profile of 96 exposed Pb smelter workers (BPb median of 51 µg/dL vs. 11 ug/dL for controls), Kirby and Gyntelberg (1985) reported that diastolic pressure was significantly associated with BPb concentrations among Pb smelter

workers, giving support to the hypothesis of a positive association between Pb exposure and high blood pressure (Kirby and Gyntelberg, 1985).   In a study by deKort et al., (1986) they evaluated the association of Pb and blood pressure in a group of 53 occupationally exposed workers (from a plant processing Pb and Cd compounds) that was compared with a control group of 52 workers not occupationally exposed (from a plant where insulation materials were produced) (deKort et al., 1988). For the results to be included in the analysis, the worker had to have been employed for more than 1 year and not be under treatment for hypertension. They reported that among workers processing Pb and cadmium compounds, BP was significantly associated with BPb levels (average BPb = 47.4 µg/dL for cases vs. 8.1 µg/dL for controls) and urinary cadmium levels (deKort et al., 1986).   Weiss et al (1988) examined the relationship of BPb to systolic and diastolic blood pressure in a longitudinal study of 89 Boston policemen (Weiss et al., 1988). Their multivariate analysis model revealed that after adjusting for previous systolic blood pressure, body mass index, age, and cigarette smoking, an elevated BPb level was a significant predictor of subsequent systolic blood pressure. Although, there are some inherent problems with the study design (BPb measured at baseline only, observations on small number of subjects, and pooling of data) their findings provided supporting evidence for the observed association, suggesting that BPb level can influence systolic blood pressure even within the "normal" range (Weiss et al., 1988).

In a cross-sectional study, Maheswaran et al (1993) examined an association between BPb and blood pressure on 809 men occupationally exposed to Pb. Pb exposure was assessed by BPb levels, ZPP levels, and years of industrial exposure to Pb. The geometric mean BPb level was 31.6 µg/dL with a range of 0 µg/dL and 98 µg/dL. They reported that unadjusted sBP increased significantly with increasing BPb levels, from 127 mmHg (95 percent CI 123.5-130.5)

in men with BPb levels < 21 µg/dL to 133 mmHg (95 percent CI 128.7-137.3) in men with BPb

levels > 50 µg/dL. However, they also noted that when sBP was adjusted for age, body mass

index, and alcohol intake, the effect of Pb was diminished to 129 mmHg and 132 mmHg in the

respective BPb categories, and it was no longer significant. There was no significant relation

between dBP and BPb with mean unadjusted diastolic pressure at 83 mmHg in both the highest

and lowest BPb categories (Maheswaran et al., 1993).

Associations of bone and BPb with blood pressure and/or hypertension have not been

consistent in prior studies. In a cross-sectional analysis by Schwartz et al., (2000), their objective

was to determine the influence of BPb, and tibial Pb on sBP and dBP and on hypertension in 543

former Pb manufacturing workers (with an average of 18 years since last occupational exposure)

(Schwartz et al., 2000). They observed that BPb was associated with systolic and diastolic blood

pressure and hypertension status (the latter in only those subjects <58 years of age). However,

tibia Pb was not associated with any of these measures. They reported that systolic blood

pressure was elevated by BPb levels as low as 5 µg/dL, leading them to speculate that Pb may

have a transient influence on blood pressure that is related to target dose levels obtained once

release of Pb from body stores has occurred (Schwartz et al., 2000). Furthermore, in a

longitudinal analysis in this same population, Glenn et al. (2003) observed that the BPb and tibia

Pb were both associated with increases in sBP. Like the Schwartz et al study (2000), they also

did not observe an association between tibia and dBP. In addition, they also did not find an

association between BPb and dBP (Glenn et al., 2003).

In a cross-sectional study of 798 Korean lead workers, Lee et al (2001) found that tibia

lead was associated with hypertension status. They hypothesized that selected genes known to

modify the toxicokinetics of lead such as vitamin D receptor (VDR) and ALAD may influence

blood pressure and hypertension risk (Lee et al., 2001). In linear regression models in Pb workers only, VDR genotype (BB and Bb vs. bb), BPb, tibia Pb, and BPb were all positive predictors of systolic blood pressure. However, on average, Pb workers with VDR-BB or Bb genotype had diastolic blood pressures that were 1.9 mm Hg higher than did Pb workers with VDR bb (Lee et al., 2001). A study of current and former workers exposed to Pb examined the association between BPb and blood pressure and reported no modification by ALAD genotype (Weaver et al., 2008). Lastly, Weaver at al, (2008) conducted a cross-sectional data in 652 current and former Pb workers in Korea. The mean BPb was 30.9 µg/dL and mean patella Pb was 75.1 mg Pb/g bone mineral. Positive association between BPb and BP was reported. Patella Pb, however did not appear to be a significant risk factor for elevated blood pressure among these study participants.

Summarizing the published work on this topic, US EPA (2006, 2012) concluded that the reviewed studies support a relationship between increased Pb exposure and increased adverse cardiovascular outcome, including increased blood pressure, increased incidence of hypertension, and cardiovascular morbidity and mortality (EPA, 2006). Their conclusion is that every doubling of BPb is associated with a ~1.0 mm Hg increased systolic and ~0.6 mm Hg increased diastolic blood pressure for blood Pb between 1 and >40 µg/dL. In addition, EPA concludes that cumulative past Pb exposure, measured by bone Pb, may be more important than present exposure in assessing cardiovascular effects of Pb exposure. Over the range of bone Pb concentration of <1.0 µg/g to 96 µg/g, every 10 µg/g increase in bone Pb was associated with an increased odds ratio for hypertension between 1.28 and 1.86, depending upon the study. Two studies measured averaged increased systolic blood pressure of ~0.75 mm Hg for every 10 µg/g increase in bone Pb concentration over a range of <1 to 52 µg/g.

Lastly, EPA states that the females often show lower Pb coefficients than males, and blacks higher Pb coefficients than whites. However, they emphasize that where these differences have been tested, they are usually are not statistically significant. The tendencies may well arise, EPA explains, in the differential Pb exposures in these strata, lower in women than in men, higher in blacks than in whites (US EPA, 2006). In a more recent review (2012), EPA stated that current epidemiologic studies supported this conclusion at lower concurrent blood Pb levels (in populations with mean BPb levels < 2 μg/dL) and added to the evidence base regarding populations potentially at increased risk (i.e., high stress, genetic variants) and regarding associations of bone-Pb levels with blood pressure and hypertension in populations with mean bone-Pb levels less than 20 μg/g (US EPA, 2012). They further state that since studies were mostly cross-sectional in design and were conducted in adults whose concurrent BPb levels are influenced both by current Pb exposures and past Pb exposures mobilized from bone, uncertainty exists over the Pb exposure conditions that contributed to the associations observed between concurrent BPb level with increased blood pressure and hypertension (US EPA, 2012)

2.8 Effects of Lead on the Renal System

Pb toxicity has been implicated in kidney disease (nephropathy). Acute and chronic nephropathy has been observed in humans. Acute nephropathy can occur during the early stages of excess Pb exposure, especially in young children. The effects of acute nephropathy can be characterized by reversible functional and morphological changes in the kidney's proximal tubular and epithelial cells. The formation of nuclear inclusion bodies (lead-protein complex), structural changes in mitochondria, and cytomegaly in the epithelial cells are some of the morphological changes of the kidneys' response to acute Pb exposure. The initial accumulation

of absorbed Pb occurs primarily in the kidneys. This takes place mainly through glomerular filtration and subsequent reabsorption and, to a small extent, through direct tubular absorption from the blood (Nolan et al., 1992). Pb may be taken up by the renal tubular epithelial cells from the basolateral side by active transport as the free ion (Nolan et al., 1992). The uptake of Pb through the renal brush border does not appear to occur via any specific carriers. The process may involve binding of Pb to non-specific surface sites on the brush border membrane, followed by internalization by endocytosis (Victery et al., 1984; Nolan et al., 1992). The functional changes in the kidneys consist of increased aminoaciduria, glucosuria, phosphaturia, and sodium excretion; decreased uric acid excretion and 1, 25-dihydroxyvitamin D synthesis; and an altered plasma angiotensin II/rennin-ratio (Berk et al., 1970).

The main function of the renal system is characterized by the glomerular filtration or active removal of unwanted wastes and simultaneous retention molecules such as water, glucose, electrolytes, and amino acids that are essential for biological processes. The measurement of glomerular filtration rate (GFR) is not considered a very good predictor of early stage kidney clinical dysfunction. Currently, there are a number of available methodologies that can be used to clinically assess GFR. Some of these methodologies include the measurement of creatinine clearance and/or serum cystatin C. Cystatin C is a protein produced by nucleated cells and is retained in the body via a glomerular filtration and subsequent reabsorption in the kidney tubules (Fried, 2009). In order to better predict early stages of kidney dysfunction, a number of new biomarkers have been utilized. Although they have not yet been fully confirmed as early predictors of clinical renal disease, urinary β2-microglobulin, and N-acetyl- β -D-glucosaminidase (NAG) have been used more recently (Fried et al., 2009).

The proximal convoluted tubules (PCT) of the kidneys are primary target of early phase Pb exposure (U.S. DOE-REIS, 1994). Formation of inclusion bodies is the characteristic structural change at this site. These bodies are composed of a Pb-protein complex that may also function as barricade for further Pb induced damage to the kidneys. The mitochondria of the PCT's epithelial cells are the site of Pb-induced structural changes. These changes are a result of the impaired energy metabolism in the mitochondria. As a result of these changes, the ability of these cells to transport amino acids, glucose, phosphates, sodium and uric acid, result in aminoaciduria, glucosuria, phosphatouria, and decreased uric acid excretion are reported (suggestive of Franconi's syndrome) (Goyer, 1985).

Prolonged exposure to Pb may cause chronic nephropathy. In contrast to the acute nephropathy, it is characterized by sparse or absence of nuclear inclusion bodies; atrophy or hyperplasia of tubular epithelial cells; and progressive interstitial, glomerular, arterial, and arteriolar sclerosis (DOE-REIS-1994). Functional characteristics of chronic nephropathy include reduced glomerular filtration rate (GFR), azotemia, proportionally greater tubular dysfunction than indicated by the decrease in glomerular filtration rate during the early stage of chronic nephropathy, and the absence of detectable tubular dysfunction accompanying the decrease in GFR at the later stages (U.S. DOE-REIS-1994).   Chronic nephropathy does not become clinically apparent until about 50% to 75% of the tubules are damaged (Goyer et al., 1985; Landrigan et al., 1989). Recent animal studies provide further evidence of plausible biological mechanisms of kidney damage, including oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis (U.S.EPA, 2012). Effects of Pb on renal endocrine function (EPO synthesis) may be more related to the "anemia" induced by lead poisoning than its overall renal effect. However, since the Pb-induced effects on renal endocrine function may be a result of

long term effects on the kidney, the following section will briefly discuss some of the important literature that addresses the overall Pb effect on the kidneys. The research on this topic includes studies of the effects of environmental Pb exposure on general populations, as well as the effect of Pb on the occupationally exposed workers.

2.8.1 General Population Studies

In a considerable number of US studies involving several National Health and Nutrition Estimates Surveys (NHANES) II and III, researchers have pointed to a link between the Pb concentrations and its impact on the kidneys (Munter et al,. 2003, 2005; Navas-Acien et al., 2009; Kim et al., 1996; Tsaih et al., 2004; Akesson, et al., 2006; Yu et al., 2004). Munter and his colleagues, examined the association between BPb and renal function in NHANES III (data from 1988-1994) (N = 15,211) among a representative sample of the civilian, non-institutionalized U.S. population with and without hypertension, age 20 years old or older (Munter et al., 2003). They considered two indices of renal function: serum creatinine (SCr) and chronic kidney disease (defined as GFR < 60 ml/min per 1.73 m^3). They reported that among persons with hypertension, the mean BPb was 4.2 µg/dL and the prevalence of elevated serum creatinine was 11.5 percent. In those without hypertension, mean BPb was 3.30 µg/dL and the prevalence of elevated serum creatinine was 1.8 percent. Among persons with hypertension, a graded association was present between higher quartiles of BPb and a higher odds ratio of both an elevated SCr, and chronic kidney disease (CKD). In contrast, among persons without hypertension, no association was present.

In their other study of NHANES III data (1999-2002), Munter and colleagues showed that those in the highest quartile for BPb ($\geq$ 2.47 µg/dL) were 2.72 times more likely to have

CKD than those in the lowest quartile for BPb (<1.06 µg/dL). Higher BPb levels were associated with a higher multivariable-adjusted odds ratios of hypertension among non-Hispanic blacks and Mexican Americans (Munter et al., 2005). In a comparable study of nearly 15,000 adults evaluated between 1999 and 2006, Navas-Acien et al., (2009), also reported reduced GFR in those with higher BPbs (> 2.4 µg/dL) as compared to those with lower BPbs (≤ 1.1 µg/dL) (adjusted OR = 1.56; 95% CI = 1.17, 2.08). Similarly, a Swedish Women's Health Study reported an association between very low BPb concentrations and reduced GFR (Akesson et al, 2006). Notably, higher urine Pb was associated with lower estimated creatinine clearance in this study (Akesson et al., 2006). Some of the greater benefits of the larger (NHANES II and III) studies are based mainly on a large sample size and comprehensive adjustments for possible confounding factors that can influence outcomes.

In addition to these surveys, findings from the Normative Aging Study (NAS) (Kim et al., 1996; Tsaih et al., 2004) have also found similar associations. Recently, the EPA calculated that the magnitude of the impact on GFR of a change in BPb from 1.1 µg/dL (the SWHS 5th percentile) to 4.5 µg/dL (the 95 percentile) would be comparable to the loss of renal function associated with an increase in body mass index (BMI) of 7 kg/m^2 or an increase in age of 4.7 years (U.S. EPA 2006). EPA's calculations put these study findings in perspective with regard to other factors that influence kidney function (remarkably low BPb concentrations with reduced GFR). Some have considered the effects of Pb on kidneys (especially at low BPb levels e.g., <10 µg/dL) to be a result of reverse causality, which seemingly increases BPb levels as a result of renal insufficiency to excrete Pb. However, the temporal relation between Pb dose and renal function decline is a critical factor in determining causality (U.S. EPA, 2006).

Two longitudinal analyses of data from the Normative Aging Study (NAS) population have been published where the association between low-lead exposure and renal function changes over time were investigated (Kim et al., 1996; Tsaih et al., 2004). In their longitudinal general population studies, Kim et al., (1996) followed 459 men who had periodic examinations conducted every 3–5 years during 1979–1994. Mean BPb and SCr levels for this cohort, at baseline, were 9.9 µg/dL and 1.2 µg/dL, respectively. After adjustment for age, body mass index, smoking, alcohol consumption, educational level, and hypertension, they reported that BPb concentration was positively and significantly associated with concurrent concentration of SCr. They noted that a log increase in BPb level predicted an increase of 7 µmol/L (0.08 mg/dL) in serum creatinine concentration, which is roughly equivalent to the increase predicted by 20 years of aging. Furthermore, they noted that the association was also significant among subjects whose BPb concentrations had never exceeded 0.48 µmol/L (10 µg/dL) throughout the study period. The age-related increase in SCr level was earlier and faster in the group with the highest-quartile levels of long-term Pb exposure than in the group with the lowest-quartile levels (Kim et al., 1996). In their prospective study, Tsaih and colleagues (2004), examined changes in renal function during 6 years of follow-up and a relation to baseline Pb levels, diabetes, and hypertension among 448 middle-age and elderly men (a subsample of the Normative Aging Study). Their cohort included generally low Pb-exposure, with mean BPb, patella lead, and tibia lead values of 6.5 µg/dL, 32.4 µg/g, and 21.5 µg/g, respectively. In addition, they observed significant associations of bone Pb (particularly tibia bone) with prospective follow-up measures and annual changes in SCr among subjects with diabetes. Increased tibia Pb levels from the midpoints of the lowest to the highest quartiles (9–34 µg/g) were associated with an increase in SCr (Tsaih et al., 2004). However, the same study found a

significant positive association between blood and tibia Pb levels and changes in serum creatinine that were observed in susceptible populations including participants who were diagnosed with diabetes and hypertension. These two studies seem to also address a "reverse causality", which impaired kidney function results in reduced elimination of Pb, thereby increasing the Pb body burden.

In a significantly smaller study by Yu et al., (2004) 121 patients who had chronic renal insufficiency, a "normal" body Pb burden, and no history of exposure to Pb were observed prospectively for 48 months (Yu et al., 2004). The primary end point was an increase in the SCr level to double the baseline value. They found that among the 121 patients, 63 patients had a "high-normal" bone Pb. Furthermore, the patients with "high normal" bone Pb also had higher BPb than the patients with "low-normal" bone Pb. The patients in the two groups differed on only on BPb and bone Pb and none of the other baseline characteristics. Their prospective study showed that even after adjustment of other co-factors, the age-related increase in SCr level was earlier and faster in the population with the highest quartile levels of long-term Pb exposure. They estimated the Pb-body burden by administering a dose of a Pb-chelating agent ($CaNa_2EDTA$) followed by a measurement of the amount of Pb excreted via urine during the next 72 hours. They observed that both $CaNa_2EDTA$-chelatable Pb and BPb at baseline were associated with significant declines in GFR during the following four years (Yu et al., 2004). Furthermore, an improvement of GFR was reported in those who received $CaNa_2EDTA$ chelation therapy but not in those who received placebo. The GFR was significantly lower in the chelated group as compared to the placebo group (Lin et al, 2003). It is not certain if the improvement in the kidney GFR function was due to direct effects of $CaNa_2EDTA$ treatment due to the resulting of Pb removal (U.S.EPA, 2012).

Most of the research on this topic involves older populations, with relatively little information on Pb exposure and renal function in young adults or children. In one study of 769 healthy young adults between 12 and 20 years of age (NHANES III), Fadrowski and his colleagues sought to evaluate the association between BPb levels and GFR (Fradowski et al., 2010). This evaluation was based on both the serum cystatin-C and SCr levels. It is important to note that serum cystatin-C appears to be less dependent than creatinine on age, gender, height and muscle mass, making a better marker of kidney function then SCr. In the fully adjusted model, they reported that a 2-fold increase in BPb levels was associated with lower cystatin C– estimated GFR among males and females. In sensitivity analyses, they evaluated the effect of adjusting for other kidney disease risk factors. They found that less than 5% of the cohort was hypertensive. Inclusion of blood pressure status in the multivariate models did not affect the results (Fadrowski et al., 2010). This suggests that studies that rely on creatinine-based estimates of kidney function might underestimate the association between GFR and BPb level. The use of a cross-sectional design for such studies can perhaps suggest the possibility of a reverse causation (i.e., kidney disease causes decreased excretion of lead) (Bellinger et al., 2011). However, this may not prove to be the case since, some prospective studies have shown that BPb levels are associated with subsequent declines in kidney function (e.g., Kim et al., 1996). Studies of Pb exposure and kidney function in even younger children, suggest that higher BPb levels are associated with increased GFR (as estimated by SCr or cystatin-C levels) (De Burbure et al., 2006). Their most interesting and consistent finding was an overall inverse relationship between SCr beta-2-microglobulin, cystatin C, and BPb, suggesting that environmental Pb induces an early renal hyperfiltration (De Burbure et al., 2006).

The common outcome in most of these studies is that seemingly low BPbs are associated with renal dysfunction in children and chronic kidney disease in adults. When compared to the occupational studies discussed in the next section, the Pb effects at extraordinarily low BPb levels in adults in the general population seem to defy credibility. However, to some degree, concurrent BPb levels measured in adulthood are likely reflective of the exposure at earlier age as measured by the area under the curve of the lifetime relationship between BPb and time since early childhood. In addition to possible current exposure, BPb measured in adulthood can also be derived from bones where Pb is stored for many years, making it plausible that those with the highest BPb levels in the NHANES studies may have had higher BPb levels earlier in life. The general population studies benefit from a variety of strengths concerning the adverse renal effects of Pb. The associations observed in the majority of these more recent studies provide strong and consistent evidence indicating that Pb is a contributor to renal dysfunction at much lower Pb exposure levels than previously thought (US EPA, 2006)

2.8.2 Occupational Studies

Studies involving occupational research on Pb induced health effects, in contrast to the above-described general population studies, have been less consistent. The conventional wisdom has been that higher levels of exposure should inflict more injury. However, this does not seem to be the case with many of the studies discussed on this topic. Whether it is the smaller size of the studies, poor statistical power, lack of adjusting for important covariates, "healthy worker effect" or some other factors, these studies seem to show a smaller Pb impact on the renal system. Regardless, a number of early occupational exposure studies reported elevated death rates from chronic kidney diseases and residual hypertensive diseases (mainly renal) among

cohorts of smelter and battery workers exposed to Pb in an occupational setting (Cooper et al., 1985). One of the early studies was a 1975 retrospective analysis of mortality conducted by Selevan et al., for a cohort of 1,987 males employed between 1940 and 1965 at a primary Pb smelter in Idaho (Selevan et al., 1985). They reported an excess in mortality from chronic renal disease (SMR = 192; confidence interval (CI) = 88-364). Also, the risk of death from renal disease increased with increasing duration of employment (e.g., after 20 years employment) where the SMR reached 392 (CI = 107-1,004) (Selevan et al., 1985). There were no BPb levels analyzed; however, the workers were exposed to airborne levels > 200 μg Pb/m^3. In addition, two groups of male Pb workers (4,519 battery plant workers and 2,300 Pb production workers), whom had been employed for at least one year during the period January 1, 1946 through December 31, 1970, were observed for mortality during the 34 years from January 1, 1947 through December 31, 1980 (Cooper et al., 1985). A greater than expected mortality rate from excess deaths from malignant neoplasm was found among the battery plant workers. Also, other hypertensive disease (mainly renal) chronic nephritis, and a group of ill-defined conditions were also reported. Among the Pb production workers the pattern was similar, with a significant number of excess deaths from other hypertensive disease, hypertensive heart disease, chronic nephritis, and ill-defined conditions. The average BPb concentration measurements were available for only a number of workers (approximately 50%) and were 79.7 μg/dL in the smelter workers and 62.7 μg/dL in the battery workers, respectively. However, for a significant portion of the cohort, BPb levels were only estimated since they were exposed prior to the initiation of monitoring programs (Cooper et al., 1985).

In the cohorts studied by both Cooper et al., and Selevan et al., (1985) the majority of reported deaths occurred among workers with 20 or more years of employment or those that had

been hired before 1946. In one of two other studies in which renal function was evaluated longitudinally in Pb workers, Coratelli et al. (1988) reported a decline in urinary N-acetyl-b-D-glucosaminidase (NAG) over a 1-month period of decreased occupational exposure in 20 Pb battery factory workers followed over a 1-year period. NAG increased when exposure resumed. However, no association was observed between NAG and BPb (mean BPb at study onset was 47.9 mg/dL) and clinical renal function measures was observed (Coratelli et al. 1988, in Weaver et al., 2009). In the other longitudinal study of Pb workers, Hsiao et al. (2001) analyzed 8 years of annual medical surveillance data in 30 Pb battery workers in Taiwan. Their study was longitudinal in design, and periodically monitored the BPb levels of 30 Pb battery workers, and they reported that a higher BPb level was associated with lower SCr (Hsiao et al., 2001).

In their 2003 research, Weaver et al. studied 803 current and former Pb workers and 135 controls in Korea in order to compare associations of Pb biomarkers with renal function in current and former Pb workers. They utilized a cross-sectional analysis of first year results from a longitudinal study of renal function by assessing blood urea nitrogen (BUN), SCr, and measured and calculated creatinine clearance. Urinary NAG and retinol-binding protein were also measured (Weaver et al., 2003). Mean tibia lead, and BPb levels in Pb workers were 37.2 μg/g (SD=40.2) and 32.0 μg/dl (SD=15.0) respectively. Their study showed that higher Pb measures were associated with worse renal function in 16/42 models and when influential outliers were removed, higher Pb measures remained associated with worse renal function in nine models (Weaver et al., 2003).

In addition, Weaver et al., (2006) assessed effect modification by polymorphisms in the genes encoding for ALAD, the vitamin D receptor (VDR), and endothelial nitric oxide synthesis on the reported associations of lead biomarkers and renal function (Weaver et al., 2006). Renal

function was assessed via blood urea nitrogen, serum creatinine, measured and calculated creatinine clearances, urinary N-acetyl-beta-D-glucosaminidase, and retinol-binding protein. Little evidence of effect modification by genotype on associations between patella Pb and renal outcomes was observed. The vitamin D receptor polymorphism did modify associations between the Pb biomarkers and SCr and calculated creatinine clearance. In those with ALAD1 genotype and higher BPb, they also observed a higher creatinine clearance, (Weaver et al, 2006).

In a more recent longitudinal study of a large population of current and former workers at 26 lead manufacturing facilities in South Korea, workers were evaluated three times, roughly one year apart, at which time BPb, tibia bone Pb, and markers of renal function were assessed (Weaver et al, 2009). In this longitudinal analysis of data from current and former Pb workers followed over a 2-year period, they utilized a generalized estimating equation (GEE) in a modeling approach in order to separate the effects of recent dose (as estimated by blood lead) from the chronic effects of cumulative dose. At baseline, mean age and duration of occupational Pb exposure were 42.0 μg/dL (SD=9.3) and 8.8 μg/dL (SD=6.3) years, respectively, in 537 current and former lead workers. Mean blood and tibia Pb were 31.3 μg/dL (SD=14.4) and 35.0 μg/g (SD=37.8) bone mineral, respectively. They found that in males, SCr decreased and calculated creatinine clearance increased over the course of the study. Mean BPb was not significantly different between evaluations 1 and 3 in either males or females. Blood and tibia Pb were significantly associated with decline in renal function. Furthermore, SCr decreases and calculated creatinine clearance increases were greatest in male participants whose BPb declined (Weaver et al., 2009).

2.9 Lead Induced Anemia

  i) Effects of Lead on Heme Synthesis and Erythrocyte Survival

  Lead has long been known to alter the hematological system. The anemia induced by Pb results primarily from both inhibition of heme synthesis and shortening of the erythrocyte lifespan. Pb interferes with heme synthesis by altering the activities of δ-aminolevulinic acid dehydratase (ALAD), ferrochelatase and other enzymes in the heme synthetic pathway. As a result of these effects, Pb-exposed individuals can present an increased urinary porphyrin, coproporphyrin, and δ-aminolevulinic acid (ALA); increased blood and plasma ALA; and increased erythrocyte protoporphyrin (EP). Studies of lead workers have shown that erythrocyte ALAD activity correlated inversely with BPb (Hernberg et al. 1970) their studies indicate that the activity of ALAD is inhibited at very low BPb, with no apparent threshold. ALAD activity was inversely correlated with BPb over the entire range of 3– 34 µg/dL in urban subjects never exposed occupationally (Hernberg and Nikkanen 1970). Pb also inhibits the enzyme pyrimidine-5'-nucleotidase within the erythrocyte, which results in an accumulation of pyrimidine nucleotides (cytidine and uridine phosphates) in the erythrocyte or reticulocyte leading to so-called basophilic stippling and subsequent destruction of these cells. This has been reported in lead workers, with the greatest inhibition and marked accumulations of pyrimidine nucleotides apparent in workers with overt intoxication, including anemia (Paglia et al. 1975, 1977); BPb levels in these workers ranged between 45 and 110 µg/dL. Pyrimidine-5'-nucleotidase activity was inversely correlated with BPb even when corrected for an enhanced population of young cells due to hemolytic anemia in some of the workers (Buc and Kaplan 1978). Erythrocyte pyrimidine-5'-nucleotidase is also inhibited in children at very low BPb. A significant negative linear correlation between pyrimidine-5'-nucleotidase and PbB level was seen in 21 children with

PbBs ranging from 7 to 80 µg/dL (Angle and McIntire 1978). Similar results were seen in another study with 42 children whose PbB ranged from <10 to 72 µg/dL (Angle et al. 1982).

There are two kinds of globular proteins embedded on the erythrocyte membrane. One is integral protein that penetrates into the lipid bilayer and is responsible for membrane ion selectivity and water transport (Singer et al, 1972). Another type is a peripheral protein that exists on the inside surface of the erythrocyte membrane and maintains the fluidity of membrane construction (Lux et al., 1979; Steck et al., 1974). Both proteins were reported to decrease in lead exposed workers (Fukomoto et al., 1983). Their decrease may lower the ability of membrane transportation, resulting in difficulty in the preservation of Na+ and water and therefore a decrease in erythrocyte (Karai et al., 1981).

The relationship of Pb exposure and anemia in terms of dose-response relationship and thresholds indexed by various exposure markers has been recorded relatively well. The older literature documents thresholds for onset of Pb derived anemia at lower levels in children (<40 µg/dL) than in adults (≥40-50 µg/dL). A number of epidemiological studies evaluated the population response for a selected Hgb reduction at varying levels of BPb in Pb workers (Table 2-5). In the U.S. for example, a dose-response relationship between BPb levels and toxic effects have been evaluated in 160 Pb workers with BPb levles of 16-280 µg/dL (Baker et al., 1979). Clinical evidence of toxic exposure was found in 70 workers (44%). The study reported that anemia (Hgb level < 14.0 g/dL) was found in 5% of workers with BPb of 40-59 µg/dL, in14% with levels of 60-79 µg/dL, and in 36% with levels >80 µg/dL (Baker et al., 1979).

Table 2-5: Selected Studies in Lead-Induced Anemia

Researcher/s	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Waldron (1966), Chisolm (1971), U.S. CDC (1978), Moore (1988)	Selected earlier studies of Lead workers and exposed children	Chronic Lead poisoning: Hgb, children: <10 g/dL; workers: <13 g/dL	>40-100 µg/dL	Lead induced reduction in Hgb, from heme and globin reduction
Pueschel et al.(1972)	540 Lead poisoned children (N=540)	Reduced Hgb levels	Blood lead, 30-120 µg/dL	Negative correlation between Hgb and BP
Grandjean (1979)	Lead workers (N=202)	BPb versus Hgb	Wide BPb range: < 25 to > 60 µg/dL	Hgb reduction: 17% ,< 25 µg/dL; 26% 26-60 µg/dL; 45% .> 60 µg/Dl
Hu et al. (1994)	Carpentry male workers (N=119)	BPb or patella bone versus Hgb	Mean Pb= 8 µg/dL	No reduction of Hgb versus BPb PbB, reduction of Hgb versus patella Pb

More recent research studies have reported mixed results for the relationship of a dose-response for Hgb reduction with increased BPb levels. It can be said that a number of the newer investigations had the benefit of much better epidemiological methods employed in these studies. They all have a relatively larger sample size, more extensive statistical controls for various confounding factors, and a methodology for better determining the relative utility of various Pb exposure markers in documenting lead effects on the hemopoietic system. In a group of carpenters whose mean BPb was 8 µg/dL, patella bone Pb levels were associated with decreased Hgb levels but BPb was not. This observation may reflect a subclinical effect of bone Pb stores on hematopoiesis and was the first epidemiological evidence that bone Pb may be an important biological marker of ongoing chronic toxicity (Hu et al., 1994).

ii) Effects of Lead on Serum Erythropoietin

In the previous section, literature that was presented that supports the conventional belief that the anemia induced by Pb is primarily the result of both inhibition of heme biosynthesis and shortening of erythrocyte life span. However, it has recently been realized that these adverse effects alone still may not completely account for the effects of Pb on hemoglobin (Hgb) levels. Pb can also induce an inappropriately low production of erythropoietin (EPO), evidence of renal endocrine dysfunction, leading to inadequate maturation of red cell progenitors, which can contribute to anemia (Patil et al., 2006). Indeed a growing number of studies have suggested that the endocrine function of the kidneys may also be affected by Pb exposure. Mainly, they suggest that Pb may inhibit the synthesis of erythropoietin (EPO), a glycoprotein hormone which regulates both steady-state and accelerated erythrocyte production (Caro et al., 1984; Anetor, et al., 2005). More than 90 percent of EPO is produced in the proximal renal tubule (PRT) (Harlan et al., 1985). Coincidentally, this is the area of the kidney where Pb tends to accumulate (Goyer et al., 1973).

EPO production is a principal factor in maintaining normal erythropoiesis (Caro et al., 1984; Erslev et al., 1986; Mushak et al., 2011). In a number of studies (Table 6), it has been shown that serum EPO levels were significantly affected by Pb primarily in adult women with elevated environmental Pb exposure (Graziano et al., 1991) and in children with elevated BPb levels (Liebelt et al., 1999; Graziano et al., 2004). In addition, the effects of Pb on EPO production were also shown in Pb workers (Osterode et al., 1999; Romeo et al., 1996), as well as in taxi drivers exposed to Pb in air (Sakata et al., 2007). The results of some of the referenced studies are summarized in Table 2-6.

Table 2-6: Studies of Lead Effects on Serum Erythropoietin

Researchers	Study Cohort	Measured Endpoint(s)	Levels of Lead Exposure	Reported Findings
Romeo et al. (1996)	Male lead workers (N=141)	Level of serum EPO versus BPb	Lower exposure BPb mean =30 μg/dl; higher exposure mean = 65 μg/dL	EPO negatively associated with BPb
Liebelt et al. (1999)	Children -community exposures (N=86) aged 1-6 years	Level of serum EPO versus BPb	BPb Median =18 μg/dl; PbB range =2-84 μg/dL	Negative association of EPO versus BPb
Kim et al. (2002)	Korean lead battery workers (N=66) and 26 control workers	P5N activity versus BPb	Mean BPb 546 μg/dl, 39/66 >40 μg/dL	Significant negative association of P5N inhibition with PbB
Graziano et al. (2004)	Yugoslavia prospective Pb study of children (N=311) aged 4..5-12 years	Level of serum EPO vs. BPb	Mean BPbs for control town: 6-9 μg/dl; mean for exposed children:31-39 μg/dL	Significant positive association of EPO vs. BPb at ages 4.5 and 6.5 but insignificant linkage at older ages
Sakata et al. (2007)	Nepalese tricycle cab drivers (N=27)	Level of serum EPO vs. BPb	Mean BPb =6.4 μg/dL	Significant negative association of EPO and BPb

One of the first studies of associations between lead and EPO took place in Kosovo. Significantly depressed serum EPO was observed among pregnant women; a negative association between BPb and EPO occurred, particularly in the low hemoglobin strata (Graziano et al., 1991). The work of Dr. Graziano's team was provoked by a simple experiment by Grandjean et al., (1989) in 25 Danish Pb workers (mean BPb = 44 ug/dL) and 25 age-matched controls who were asked to donate a unit (450 ml) of blood. After monitoring hemoglobin and reticulocyte counts over time, the controls demonstrated a prompt reticulocytosis and return to the baseline hemoglobin level. However, it took the Pb workers roughly two weeks longer to return to the baseline hemoglobin levels. The authors attributed this finding to the Pb induced impairment of heme synthesis. However, the reported effect was later attributed to Pb-induced impairment of EPO production as shown in an environmentally exposed population of pregnant

women (Graziano et al., 1991). This study selected a group of pregnant women that included

those with the highest and with the lowest BPbs within four strata of Hgb levels (9-9.9, 10-10.9,

11-11.9, 12-12.9 g/dl). After controlling for Hgb, a strong predictor of EPO, they found that BPb

was inversely related to serum EPO at both mid-pregnancy and at delivery. As indicated in the

Figure 2-9, they observed that in the women with low Hgb levels, serum EPO was inversely

associated with BPb.
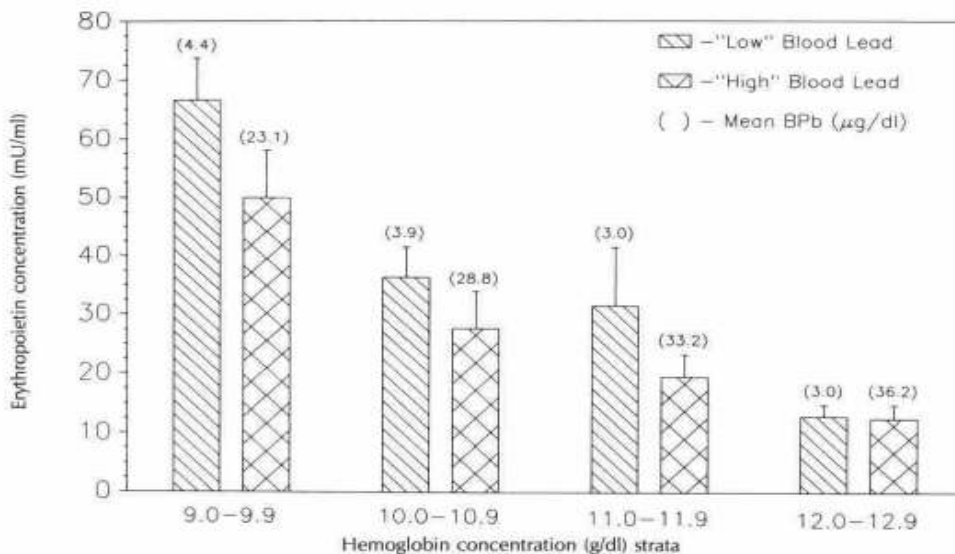
Figure 2-9: Combined EPO findings of independent experiments at mid-pregnancy (Table 1) and at delivery (Table 2). Each bar represents the mean (± SD) serum EPO concentrations of a group of 12 woman (6 at mid-pregnancy and 6 at delivery) whose concurrent hemoglobin concentrations were within indicated stratum. The mean blood lead (BPb) of each group is shown in parentheses. Source: Graziano et al., (1991). Archives of Environmental Health 46: 347-350.

Following this finding, the same group examined the association between BPb and EPO

in children in their cohort at ages of 4.5, 6.5, 9.5 and 12.5 years. They found that, while the

children with elevated BPbs maintained a normal Hgb, they required a hyperproduction of EPO

to do so (Graziano et al., 2004). Thus, in contrast to their findings in pregnant women, this

follow up study among the children showed that BPb was initially *positively* related to serum

EPO at ages 4.5 and 6.5 years. However, this trend was not seen later in their lives. At ages 9.5

and 12.5 among the same children, they started to observe that there was no positive association

between serum EPO and BPb. In fact, as the children's age increased, the researchers observed a rotation of the slope of the relationship between serum EPO and BPb. Thus, study found significant positive associations of serum EPO levels with BPb at ages 4.5 and 6.5 years, but no such association as these children got older. The decrease in EPO concentration with increase in age (up to the age of 12.5), led to the conclusion that non-anemic Pb exposed young children required hyperproduction of EPO to maintain normal levels of Hgb concentrations in blood (Graziano et al., 2004). However, the decline in slope of the relationship between EPO and BPb with age, (Figures 8 and 9) suggests that children with elevated BPb have a decreased capacity to produce EPO over the course of time. This decline is likely the result of cell damage in the proximal tubule (PRT), the site of EPO production and Pb toxicity (Graziano et al., 2004). This finding provided confidence in the hypothesis that in non-anemic Pb-exposed young children, increased erythrocyte production is required in order to maintain normal hemoglobin concentration (Graziano et al., 2004).

Another study that examined the relationship between serum EPO and BPb levels is that of Liebelt et al., (1999) who found a negative association between EPO and BPb in 86 children, ranging from 1 to 16 years of age. The mean age of the 86 children used in the final analysis was 34 months, with 55 boys and 31 girls making up the cohort. Twenty-two children had BPb 10 μg/dL, 27 had BPb between 10 and 19 μg/dL), 26 had BPb between 20 and 34 μg/dL), and 14 had BPb ≥ 35 μg/dL). The mean EPO concentration of children in the lowest Hgb stratum was compared with the mean EPO concentration in the highest Hgb stratum. Analysis of variance demonstrated a significant BPb effect, as well as a significant Hgb effect. They also reported a significant negative association between BPb in children and EPO concentrations, independent of the effects of Hgb.

CHAPTER III: POWER CALCULATIONS

BPb measures were available for each subject from birth through 12.5 years of age, and at the follow up at ~ 25 years of age. At age 12.5, the mean BPb concentrations were 30.6 μg/dl in Mitrovica, and 6.1 μg/dl in Prishtina. When examining associations between health outcomes and Pb exposure, researchers have typically used alternative ways to determine the most critical period of exposure. Of course, we examined the association with concurrent BPb. However, we also examine the associations between health outcomes and the total area under the BPb vs age curve (AUC) (using the trapezoidal method) for early post-natal (birth to age 12.5) periods. In this manner we attempted to determine a critical time period of exposure with regard to each health outcome. Outcome measures included: a) blood pressure; b) serum sICAM1 and sVCAM1; and c) serum erythropoietin. For each outcome, we developed a core model including all potential determinants of outcome. Linear regression was used to model associations between BPb and BP, sICAM-1and sVCAM-1 controlling for ethnicity, gender, body mass index (BMI), smoking, employment status, and education. Potential confounders were retained in the final models if the estimated coefficient relating PBb to BP, sICAM-1 and sVCAM-1 changed at least 10% with their inclusion.

Power was calculated using PS Power and Sample Size Program as described by Dupont and Plummer (Dupont et al., 1990). The study sample was made up of 101 participants from the former "Yugoslavia Birth Cohort" (80 experimental subjects in Mitrovica and 21control subjects in Prishtina).

3-1 Power Calculations for Specific Aim # 1

Hypothesis #1 and #2: Higher levels of prenatal and early adulthood Pb exposure are associated with increase in BP and increased levels of the circulating sICAM-1 and sVCAM-1.

All BP measurements were repeated to insure the reliability of the results. This was achieved by measuring the BP three times (one minute apart). The first measurements were dropped and we used the average for the remaining two measurements. All significance testing was two-sided with 95% confidence intervals. A previous study of the entire cohort detected a small increase in the BP at age 5.5 years old (Factor-Litvak et al., 1996). Their mean BP at that time was 100.5 (10.7) in Mitrovica and 98.4 (10) in Prishtina.

a) Systolic Blood Pressure

The measured results within each subject group were normally distributed with standard deviation 11.2. The true difference in the experimental and control means was 4.6, and we were able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.377. The Type I error probability associated with this test of this null hypothesis is 0.05.

b) Diastolic Blood Pressure

The measured results within each subject group were normally distributed with standard deviation 5.8. The true difference in the experimental and control means was 2.33, and we were able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.363. The Type I error probability associated with this test of this null hypothesis is 0.05.

The impact of environmental Pb on the cardiovascular system was explored utilizing a multiple linear regression analysis while adjusting for covariates such as body mass index, age, cigarette smoking. Thus we were able to evaluate the associations of BPb levels, and levels of sICAM-1 and sVCAM-1 markers of vascular inflammation and endothelial dysfunction that are detectable in the circulation. We expected to see increasing levels of sVCAM-1 and sICAM-1 with increasing BPb levels. Our study sample included 80 experimental subjects and 21 control subjects.

c) sVCAM-1

The measured results within each subject group were normally distributed with standard deviation 203. The true difference in the experimental and control means was 75, and we were able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.315.   The Type I error probability associated with this test of this null hypothesis is 0.05.

d) sICAM-1

The measured results within each subject group were normally distributed with standard deviation 50.5. The true difference in the experimental and control means was 18.4, and we were able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.309.   The Type I error probability associated with this test of this null hypothesis is 0.05.

3-2 Power Calculation for Specific Aim # 2:

Hypothesis 3: Higher levels of prenatal and early adulthood Pb exposure are associated with a decline in the slope of the relationship between EPO and BPb with age (after adjusting for Hgb), suggesting that individuals with elevated BPb early in life have a decreased capacity to produce EPO over time.

      The association between BPb and EPO was examined using regression analysis. First, we evaluated the association between BPb and EPO at each age using linear regression analysis, controlling for concurrent Hgb concentration. Concurrent Hgb was controlled because it is the most important predictor of EPO (Factor-Litvak et al., 1998). Second, we combined data from all ages and use repeated measures analysis to determine whether the associations between BPb and EPO, controlling for Hgb, have changed over time (Factor-Litvak et al., 1998). We used the cumulative life time exposure (including concurrent) BPb from birth to current age.

      Erythropoietin

      The measured results within each subject group were normally distributed with standard deviation 5.6. The true difference in the experimental and control means was 2.5, and we were able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.433. The Type I error probability associated with this test of this null hypothesis is 0.05.

REFERENCES (Chapter I, II, & III)

Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead. Atlanta, GA: US Department of Health and Human Services, Public Health Service, 2007.

Ahamed, M, Verma, S, Kumar, A, Sidiqui, MKJ. 2006. Delta-aminolevulinic acid dehydratase inhibition and oxidative stress in relation to blood lead among urban adolescent. Human Exposure Toxicology. 25: 547–553.

Akesson, A.; Lundh, T.; Vahter, M.; Bjellerup, P.; Lidfeldt, J.; Nerbrand, C.; Goran, S.; Strömberg, U.; Skerfving, S. 2005. Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. Environ. Health Perspectives; 113: 1627-1631.

Alexander FW, Clayton BE and Delves HT. 1974. Mineral and trace-metal balances in children receiving normal and synthetic diets. Quarterly Journal of Medicine. 43, 89-111.

Angle CR, McIntire MS. 1978. Low level lead and inhibition of erythrocyte pyrimidine nucleotidase. Environ Res 17:296-302.

Angle CR, McIntire MS, Swanson MS, et al. 1982. Erythrocyte nucleotides in children--increased blood lead and cytidine triphosphate. Pediatr Res 16:331-334.

Aufderheide, AC; Wittmers, LE, Jr. 1992. Selected aspects of the spatial distribution of lead in bone. Neurotoxicology 13: 809-819.

Baker EL Jr, Landrigan PJ, Barbour AG, et al. 1979. Occupational lead poisoning in the United States: clinical and biochemical findings related to blood lead levels. Br J Ind Med. 36:314–322

Barry, P.S.I. & Mossman, D.B. 1970. Lead concentrations in human tissues. British journal of industrial medicine. 27: 339–351.

Barry, P.S.I. 1981. Concentrations of lead in the tissues of children. British journal of industrial medicine. 38: 61–71.

Bellinger DC, Stiles KM, Needleman HL. 1992. Low-level lead exposure, intelligence and academic achievement: a long term follow-up study. Pediatrics 90:855–861.

Bellinger, D, Dietrich, K. 1995. Low-level lead exposure and cognitive function in children, Pediatr. Ann. 23 600–605.

Bellinger, D. 2011. The Protean Toxicities of Lead: New Chapters in a Familiar Story Int. J. Environ. Res. Public Health. 8: 2594-2628

Bergdahl, I.A., Grubb, A., Schutz, A., Desnick, R.J., Wetmur, J.G., Sassa, S., et al. 1997. Lead binding to δ-aminolevulinic acid dehydratase (ALAD) in human erythrocytes. Pharmacology Toxicology. 81, 153-158.

Bergdahl I.A., Sheveleva M., Schütz A., et al. 1998. Plasma and blood lead in humans: capacity-limited binding to delta-aminolevulinic acid dehydratase and other lead-binding components. Toxicological Science. 46: 247–53.

Berk PD, Tschudy DP, Shepley LA, Waggoner JG, Berlin NI. 1970. Hematologic and biochemical studies in a case of lead poisoning. American Journal of Medicine. 48:137-144.

Brenard, SR. 1977. Dosimetric data and metabolic model for lead. Health Physics, 32: 44-46.

Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, et al. 2001. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 104:1336–1342.

Blann, A.D., Nadar, S.K. and Lip, G.Y. 2003. The adhesion molecule P-selectin and cardiovascular disease. Eur. Heart J., 24(24):2166–79.

Bradberry S., Vale A. 2007. Specific substances leas and mercury. Medicine. 35:12: 627-628.

Brown CJ, Chenery SRN, Smith B, Tomkins A, Roberts GJ, Serunjogi L, et al. 2002. Sampling and analytical methodology for dental trace element analysis. Analyst 127:319–323.

Brito JAA, McNeill FE, Webber CE, Wells S, Richard N, Carvalho ML, et al. 2002. Evaluation of a novel structural model to describe the endogenous release of lead from bone. J Environ Monit 4:194–201.

Buc HA, Kaplan JC. 1978). Red-cell pyrimidine 5'-nucleotidase and lead poisoning. Clin Chim Acta 87:49-55.

Canfield, R.L., Henderson, C.R., Cory-Slechta, D.A., Cox, C., Jusko, T.A., & Lanphear, B.P. 2003. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. New England Journal of Medicine, 348, 1517–1526.

Carlos, T. M. and Harlan, J. M. 1994. Leukocyte-endothelial adhesion molecules. Blood 84, 2068-2101.

Caro J, Erslev AJ. 1984. Biologic and immunologic erythropoietin in extracts from hypoxic whole rat kidneys and their glomerular and tubular fractons. Journal of Laboratory and Clinical Medicine. 103:922-931.

CDC- Centers for Disease Control and Prevention. Third National Report on Human Exposure to Environmental Chemicals. NCEH Pub. No. 05-0570, Lead CAS No. 7439- 92-1. CDC, Atlanta, Georgia, USA; 2005a

CDC-Centers for Disease Control and Prevention. Preventing Lead Poisoning in Young Children CDC, Atlanta, Georgia, USA; 1991

Chae CU, Lee RT, Rifai N, Ridker PM. 2001. Blood pressure and inflammation in apparently healthy men. Hypertension. 38(3):399-403.

Chen, A; Rhoads,G; Cai, B; Salganik, M; Rogan, W. 2006. The Effect of Chelation on Blood Pressure in Lead-Exposed Children: A Randomized Study. Environmental Health Perspectives 114, 4, 579-583.

Chen, Y; Santella, R; Kibriya M; Wang, Q; Kappil, M; Verret, M; Graziano, J; Ahsan H. 2007. Association between Arsenic Exposure from Drinking Water and Plasma Levels of Soluble Cell Adhesion Molecules. Environmental Health Perspectives; 115, 10, 1415-1420|

Cheng Y, Schwartz J, Sparrow D, et al. 2001. Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension: the Normative Aging Study. American Journal of Epidemiology. 153:164–71.

Chisolm Jr., J.J. Increased lead absorption: toxicological considerations. 1971. Pediatrics. 48, 349-352.

Cooper, C.W.; Gaffey, W.R. Mortality of lead workers. 1985. J. Occup. Med; 17(2):100-107.

Coratelli, P; Giannattasio, M; Lomonte, C; Marzolla, R; Rana, F; L'Abbate, N. 1988. Enzymuria to detect tubular injury in workers exposed to lead: a 12-month follow-up. Contrib Nephrol. 68:207–211.

Cowley Jr AW, Roman RJ. 1996. The role of the kidney in hypertension. JAMA. 275:1581-1589.David C. Bellinger.

De Burbure, C.; Buchet, J.P.; Leroyer, A.; Nisse, C.; Haguenoer, J.M.; Mutti, A.; Smerhovsky, Z.; Cikrt, M.; Trzcinka-Ochocka, M.; Razniewska, G.; Jakubowski, M.; Bernard, A. 2006. Renal and neurological effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ. Health Perspect. 115, 584-590.

deKort, W.L.A.M.; Verschoor, M.A.; Wibowo, A.A.E.; van Hemmen, J.J. 1987. Occupational exposure to lead and blood pressure. A study of 105 workers. Am. J. Ind. Med; 11(2):145-56.

Delves, H.T., B.E. Clayton, A. Carmichael, M. Bubear, and M. Smith. 1982. An appraisal of the analytical significance of tooth-lead measurements as possible indices of environmental exposure of children to lead. Ann. Clin. Biochem. 19:329 337.

Den Hond, E.; Nawrot, T.; Staessen, J. A. 2002. The relationship between blood pressure and blood lead in NHANES III. J. Hum. Hypertens. 16: 563-568.

Dietrich KN, Succop PA, Berger OG, Hammond PB, Bornschein RL. 1991. Lead exposure and the cognitive development of urban preschool children: the Cincinnati Lead Study Cohort at age 4 years. Neurotoxicology and Teratology. 13:203-211.

Dudek, B. and Mercez, D. 1997. Impairment of physiological function in children environmentally exposed to lead. International Journal of Occupational Medicine and Env. Health. 10 (1) 37-46.

Dursun, N; Arifoglu, C; Suer, C; Keskinol, L. 2005. Blood pressure relationship to nitric oxide, lipid peroxidation, renal function, and renal blood flow in rats exposed to low lead levels. Biol Trace Elem Res 104: 141-149.

Dupont, WD; Plummer Jr.,WD. 1998. Power and sample size calculations: A review and computer program, Department of Preventive Medicine, Vanderbilt University of School of Medicine, Nashville, Tennessee, USA

Elwood, P.C.; Davey-Smith, G.; Oldham, P.D.; Toothill, C. 1988a. Two Welsh surveys of blood lead and blood pressure. Environ. Health Perspect. 78:119-121.

Elwood, P.C. Yarnell, J.W.G.; Oldham, P.D.; et al. 1988b. Blood pressure and blood lead in surveys in Wales. Am. J. Epidemiol. 127:942-945.

Ernhart CB., Morrow-Tlucak, M., .Wolf AW., Super, D., Drotar, D. 1989. Low level lead exposure in the prenatal and early preschool periods: Intelligence prior to school entry. Neurotoxicology and Teratology; Volume 11, Issue 2, Pages 161–170

Erslev AJ, Caro J. 1986. Physiologic and molecular biology of erythropoietin. Med Oncology Tumor Pharmacother 3:159-164

Factor-Litvak, P, Graziano, JH, Stein, Z. 1993. Increased risk of proteinuria among a cohort of lead-exposed pregnant women. Environmental Health Perspectives 101: 418-421.

Factor-Litvak, P, Kline, J, Popovac, D, Hadzialjevic, S, Lekic, V, Preteni-Redjepi, E, Capuni-Paracka, S, Slavkovich, V, Graziano, JH. 1996. Blood lead and blood pressure in young children. Epidemiology. 7: 633-637.

Factor-Litvak, P, Slavkovich, V, Liu, L, Popovac, D, Preteni, E, Capuni-Paracka, S, Hadzialjevic, S, Lekic, L, LoIacono, N, Kline, J, and Graziano, JH. 1998. Hyperproduction of erythropoietin in non-anemic lead-exposed children. Environmental Health Perspectives. 106; 6, 361-364.

Factor-Litvak, P, Wasserman, G, Kline, J, Graziano, JH. 1999. The Yugoslavia prospective study of environmental lead exposure. Environmental Health Perspectives. 107: 9-15.

Fadrowski, J.J.; Navas-Acien, A.; Tellez-Plaza, M.; Guallar, E.; Weaver, V.M.; Furth, S.L. ; (2010). Blood lead level and kidney function in US adolescents. Arch. Intern. Med. 170, 75-82.

Flegal AR, Smith DR. 1995. Measurements of environmental lead contamination and human exposure. Rev Environ Contam Toxicol. 143:1-45.

Fleming, D. E. B.; Chettle, D. R.; Wetmur, J. G.; Desnick, R. J.; Robin, J.-P.; Boulay, D.; Richard, N. S.; Gordon, C. L.; Webber, C. E. 1998. Effect of the delta-aminolevulinate dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. Environ. Res. 77: 49-61.

Fried, LF. 2009. Creatinine and cystatin C: what are the values? Commentary Kidney International 75, 578-580

Fujivara,C, Kaji, T, Yamamoto, C, Sakamoto, M, Kozuka, H. 1995. Stimulatory effect of lead effect of lead on the proliferation of cultured vascular smooth-muscle cells. Toxicology 98: 105-110.

Fukumoto, K, Karai, I and Horiguchi, S. 1983. Effect of Pb on erythrocyte membrane. British Journal of Industrial Medicine, 40, 220-223

Glenn, B. S.; Stewart, W. F.; Links, J. M.; Todd, A. C.; Schwartz, B. S. 2003. The longitudinal association of lead with blood pressure. Epidemiology 14: 30-36.

Goodwin HA. 2001.The biological chemistry of lead. Current Opinion in Chemical Biology; 5: 223–27.

Goyer, R.A., and B.C. Rhyne. 1973. Pathological effects of lead. Int. Rev. Exp. Pathol. 12:1 77.

Goyer, R.A. 1985. Renal changes associated with lead exposure. In: Dietary and Environmental Lead: Human Health Effects. K.R. Mahaffey, Ed., Elsevier, New York, pp. 315-338.

Grandjean, P. 1979. Occupational lead exposure in Denmark: screening with the hematofluorometer. Br. J. Ind. Med. 36, 52_58.

Grandjean P, Jensen BM, Sando SH, Jogensen PJ, Antonsen S. 1989. Delayed blood regeneration in lead exposure: an effect on reserve capacity. Am J Public Health 79:1385-1388).

Grandjean, P., Hollnagel, H., Hedegaard, L., Christensen, J.M., Larsen, S. 1989. Blood lead blood pressure relations: alcohol intake and hemoglobin as confounders. Am. J. Epidemiol. 129, 732-734.

Grant, L.D. 2009. "Lead and compounds". In Lippmann, M.. Environmental Toxicants: Human Exposures and Their Health Effects (3rd ed.). Wiley-Interscience.

Graziano, JH, Popovac, D, Factor-Litvak, P, Shrout, P, Kline, J, Murphy, M, Zhao, A, Mehmeti, A, Ahmedi, X, Rajovic, B et al. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environ Health Perspectives 89: 95-100.

Graziano, JH, Slavkovic, V, Factor-Litvak, P, et al. 1991.Depressed serum erythropoietin in pregnant women with elevated blood lead. Archives of Environmental Health 46: 347-350.

Graziano, JH, Slavkovich, V, Liu, X, Factor-Litvak, P, Todd, A. 2004. A prospective study of prenatal and childhood lead exposure and erythropoietin production. Journal of Occupational and Environmental Medicine 46: 924-929.

Greenberg, A, Parkinson, D, Fetterolf, D, Puschett, J. Ellis, K, Wielopolski, L, Vaswani, A, Cohn, S, Landrigan, P. 1986. Effects of elevated lead and cadmium burdens on renal function and calcium metabolism. Archives of Environmental Health 41: 2: 69-76.

Grizzo, LT; Cordellini, S. 2008. Perinatal lead exposure affects nitric oxide and cyclooxygenase pathways in aorta of weaned rats. Toxicol Sci 103: 207-214.

Gross, S.B., E.A. Pfitzer, D.W. Yeager, and R.A. Kehoe. 1975. Lead in human tissues. Toxicol. Appl. Pharmacol. 32:638 651.

Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Korsch MJ, Vimpani G. 1997a. Pregnancy increases mobilization of lead from maternal skeleton. J Lab Clin Med 130:51–62.

Gump, BB; Stewart, P; Reihman, J; Lonky, E; Darvill, T; Matthews, KA; Parsons, PJ. 2005. Prenatal and early childhood blood lead levels and cardiovascular functioning in 9 1/2 year old children. Neurotoxicol Teratol 27: 655-665.

Harlan, W. Landis, J, Schmouder, R, Goldstein,G, Harlan, L. 1985. Blood lead and blood pressure: relationship in the adolescent and adult US population. JAMA. 253: 530–534.

Harlan, W. 1988. The relationship of blood lead levels to blood pressure in the US population. Environmental Health Perspectives 78: 9–13.

Havlik RJ, Garrison RJ, Feinleib M, et al. 1980. Evidence for additional blood pressure correlates in adults 20-56 years old. Circulation. 61: 710-5.

Hernberg S, Nurminen M, Hasan J. 1967. Nonrandom shortening of red cell survival times in men exposed to lead. Environ Res. 1:247–261.

Hernberg S, Nikkanen J. 1970. Enzyme inhibition by lead under normal urban conditions. Lancet 1:6364.

Hernberg S, Nikkanen J, Mellin G, et al. 1970. δ-Aminolevulinic acid dehydrase as a measure of lead exposure. Arch Environ Health 21:140-145.

Hernandez-Avila M, Smith D, Meneses F, Sanin LH & Hu, H. 1998. The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. Environmental Health Perspectives 106(8):473-47

Hertz-Picciotto I, Croft J. 1993. Review of the relation between blood lead and blood pressure. Epidemiology Rev.15: 352–73.

Hsiao CY, Wu HD, Lai JS, Kuo HW. 2001. A longitudinal study of the effects of long-term exposure to lead among lead battery factory workers in Taiwan (1989–1999). Sci Total Environ. 279:151–158.

Hu H. 1991. Knowledge of diagnosis and reproductive history among survivors of childhood plumbism. Am J Public Health 81:1070-1072.
Hu, H., Watanabe, H., Payton, M., Korrick, S., Rotnitzky, A. 1994. The Relationship Between Bone lead and Hemoglobin. JAMA. 272 (19):1512-1517

Hu H, Aro A, Payton M, et al. 1996. The relationship of bone and blood lead to of bone and blood lead to hypertension. The Normative Aging Study. JAMA 275:1171-6.

Hu, H.; Watanabe, H.; Payton, M.; Korrick, S.; Rotnitzky, A. 1994. The relationship between bone lead and hemoglobin. JAMA J. Am. Med. Assoc. 272: 1512-1517.

Hu, H., Shih, R., Rothenberg, S., Schwartz, B. 2007. The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. Environmental Health Perspectives. 115 (3).

Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, GottoAM Jr, et al. 1997. Circulating adhesion molecules VCAM-1,ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 96:4219–4225.

Kang G , H.K. et al. 1983.Determination of blood-lead elimination patterns of primary lead smelter workers. Journal of toxicology and environmental health, 11: 199–210.

Karai, I. and Fukumoto, K. and Horiguchi, S. 1981. Improvement in the atomic absorption determination of lead in blood. Journal of Applied Toxicology. 295-296

Khan, D, Qayyum, S, Saleem, S, Khan, F. 2008. Lead-induced oxidative stress adversely affects health of the occupational workers. Toxicology and Industrial Health, 24: 611-618.

Kim R, Rotnitzky A, Sparrow D, et ál. 1996. A longitudinal study of low-level lead exposure and impairment of renal function. The normative aging study. JAMA 275:1177-81.

Kim Y, Lee H, Lee CR, Park DU, Yang JS, Park IJ, et al. 2002. Evaluation of lead exposure in workers at secondary lead smelters in South Korea: with focus on activity of erythrocyte pyrimidine 5'nucleotidase (P5N). Sci Total Environ; 286:181– 9.

Kirby,H.; Gyntelberg, F. 1985. Blood pressure and other cardiovascular risk factors of long-term exposure to lead. Scand. J. Work Environ. Health. 11:15-19. (cited in EPA, 1986e).

Kopp SJ, Barron JT, Tow JP. 1988. Cardiovascular actions of lead and relationship to hypertension: a review. Environ Health Perspect. 78:91-99.

Korrick SA, Hunter DJ, Rotnitzky A, et al. 1999. Lead and hypertension in a sample of middle-aged women. American Journal of Public Health. 89:330–5.

Kosnett M.J. 2005. "Lead". In Brent, J. Critical Care Toxicology: Diagnosis and Management of the Critically Poisoned Patient. Gulf Professional Publishing.

Kosnett, M.J.. "Lead". In Olson, K.R. 2006. Poisoning and Drug Overdose (5th ed.). McGraw-Hill Professional.

Krauss, T, Emons, G, Kuhn, W, Augustin, H. 2002. Predictive value of routine circulating soluble endothelial cell adhesion molecule measurements during pregnancy. Clinical Chemistry 48: 9, 1418–1425.

Lakatta EG, Levy D. 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 107:139-146.

Landis, J.R.; Flegal, K.M. 1988. A generalized Mantel-Haenszel analysis of the regression of blood pressure on blood lead using NHANES II data. Environ. Health Prospect. 78:35-41.

Landrigan PJ. 1989. Toxicity of lead at low dose. Br J Ind Med. 46(9):593–596.

Lee, B.-K.; Lee, G.-S.; Stewart, W. F.; Ahn, K.-D.; Simon, D.; Kelsey, K. T.; Todd, A. C.; Schwartz, B. S. 2001. Associations of blood pressure and hypertension with lead dose measures and polymorphisms in the vitamin D receptor and δ-aminolevulinic acid dehydratase genes. Environ. Health Perspect. 109: 383-389.

Liebelt, E.L., Schonfeld, D.J., Gallagher, P. 1999. Elevated blood lead levels in children are associated with lower erythropoietin concentrations. Journal of Pediatricds 134, 107-109.

Lin JL, Lin-Tan DT, Hsu KH, Yu CC. 2003. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. N Engl J Med 348:277–286.

Lux, S.E. 1979. Dissecting the red cell membrane skeleton. Nature, 281, vol.5731,No.pp.426-429.

Maheswaran, R.; Gill, J.S.; Beevers, D.G. 1993. Blood pressure and industrial lead. Am. J. Epidemiol. 137:645-653.

Malvezzi CK, Moreira EG, Vassilieff I, Vassilieff VS, Cordellini S. 2001. Effect of L-arginine, dimercaptosuccinic acid (DMSA) and the association of L-arginine and DMSA on tissue lead mobilization and blood pressure level in plumbism. Braz J Med Biol Res 34:1341–1346.

Markowitz M. 2000. Lead poisoning. Pediatric Review 21, 327-335.

Martin, D, Glass, T., et al. 2006. Association of blood lead and tibia lead with blood pressure and hypertension in a community sample of older adults. American Journal of Epidemiology. 163: 467-478.

Menke A, Muntner P, Batuman V, Silbergeld EK, Guallar E. 2006. Blood lead below 0.48 {micro}mol/L (10 {micro}g/dL) and mortality among US adults. Circulation. 114:1388–1394.

McMichael, A, Baghurst, P, Wigg, N, Vimpani, G, Robertson, E, Roberts, R. 1988. Port Pirie cohort study: environmental exposure to lead and children's abilities at age of four years. New England Journal of Medicine 319: 488-475.

Mervaala EM, Müller DN, Park JK, Schmidt F, Löhn M, Breu V, Dragun D, Ganten D, Haller H, Luft FC. 1999. Monocyte infiltration and adhesion molecules in a rat model of high human renin hypertension. *Hypertension* 33: 389.

Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, et al. 2004. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension. 43:1239–1245.

Mohammad, A; Ali, N; Reza, B; Ali, K. 2010. Effect of ascorbic acid supplementation on nitric oxide metabolites and systolic blood pressure in rats exposed to lead. Indian J Pharmacol 42: 78-81.

Morisaki, N. 1997. New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hyper-cholesterolemia and ischemic heart disease. Atherosclerosis 131: 1, 43-48.

Moore, M.R. 1985. Uptake of lead from water. Adv. Mod. Environ. Toxicology. 9:259 270.

Munter P, He J, Vupputuri S, Coresh J, Batuman V. 2003. Blood lead and chronic kidney disease in the general United States population: Results from NHANES III . Kidney International. 63: 1044 -1050.

Munter, P, Menke, A, DeSalvo K, Rabito, F, Batuman, V. 2005. Continued decline of blood lead levels among adults in the US: The National Helath and Nutrition Examination Survey. Archives of Internal Medicine. 165: 2155-2161.

Murphy, MJ, Graziano, JH, Popovac, D, Kline, J, Mehmedi, A, Factor-Litvak, P, Ahmedi, G, Shrout, P, Rajovic, B, Nenezic, DU, Stein, ZA: Past Pregnancy Outcomes among Women Living in the Vicinity of a Lead Smelter in Kosovo, Yugoslavia. Amer J Pub Health 80: 33-35, 1990.

Mushak, P. Lead and Public Health: Science, Risk and Regulation, Chapter 16, 2011, Elseveir, Volume 10.

Nash, D.; Magder, L.S.; Sherwin, R.; Rubin, R.J.; Silbergeld, E.K. 2003. Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women. JAMA, 289, 1523-1532.

Navas-Acien, A, Guallar, E, Silbergeld, E, Rothenberg, S. 2007. Lead Exposure and Cardiovascular Disease—A Systematic Review. Environmental Health Perspectives 115: 3, 472-82.

National Research Council. 2012. Potential Health Risks to DOD Firing-Range Personnel from Recurrent Lead Exposure. National Academies Press, Washington DC.

Nawrot TS, L Thijs1, EM Den Hond, HA Roels and JA Staessen. 2002. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis Journal of Human Hypertension. 16, 123–131.

Needleman, H. et al. 1979. Deficits in psychological and classroom performance of children with elevated dentine lead levels. The New England Journal of Medicine: 300, 13, 689-695.

Needleman H, Schell A, Bellinger D, et al. 1990. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. New England Journal of Medicine. 322: 83-88.

Nolan C.V., Shaikh Z.A. 1992. Lead nephrotoxicity and associated disorders: biochemical mechanisms. Toxicology. 73 127- 146.

Nowack R, Wiecek A, Exner B, Gretz N, Ritz E. 1993. Chronic lead exposure in rats: effects on blood pressure. Eur J Clin Invest. 23:433-443.

Occupational Safety and Health Administration (OSHA) Lead Regulations. 29 CFR 1910.1025 Occupational Exposures to Lead.

O´Flaherty EJ. 1995. Physiologically based models for bone-seeking elements. V: Lead absorption and disposition in childhood. Toxicol Appl Pharmacol 131: 297–308.

Osterloh JD., Selby JV., Bernard BP., Becker CE., Menke DJ., Tepper E., Ordonez JD., Behrens B. 1989. Body burdens of lead in hypertensive nephropathy. Arch Environ Health. 44(5):304-10.

Osterode, W., Barnas, D., Geissler, K. 1999. Dose dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead: new aspects of anaemia induced by lead. Occup. Environ. Med. 56, 106-109.

Paglia DE, Valentine WN, Dahlgren JG. 1975. Effects of low level lead exposure on pyramidine-5-nucleosidase and other erythrocyte enzymes. Possible role of pyramidine-5-nucleosidase in the pathogenesis of lead-induced anemia. J Clin Invest. 56:1164–1169.

Palca, J. 1991. Get the lead-out guru challenged. Science, Vol 253; # 5022

Patil, A.J, Bhagwat, VR, Patil, JA, Dongre, NN, Ambekar, JG and Das, KK. 2006. Biochemical aspects of lead exposure in silver jewelry workers in Western Maharashtra (India). Journal of Basic and Clinical Physiology and Pharmacology 17(4), 213-229

Patrick, L. 2006. "Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment". Alternative medicine review: a journal of clinical therapeutic 11 (1): 2–22.

Pearlstein, T., Weuve, J., Schwartz, J., Sparrow, D., Wright, R., Litonjua, A., Nie, H., Hu, H. 2007. Cumulative community-level lead exposure and pulse pressure: The normative aging study. Environ. Health Perspect 115 (12), 1696–1700.

Piomelli S. et al. 1973. A micro-method for free erythrocyte porphyrins: The FEP test. Journal of Laboratory and Clinical Laboratory 81: 932-940.

Piomelli S. et al. 1982. Threshold for lead damage to heme synthesis in urban children. Proceedings of the national Academy of Sciences, USA, 79 3335-3339.

Pirkle, J, Schwartz, J, Landis, J, Harlan, W. 1985. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. American Journal of Epidemiology 121: 246–258.

Pocock SJ, Shaper AG, Ashby D, Delves T, Whitehead TP. 1984. Blood lead concentration, blood pressure, and renal function. BMJ (Clin Res Ed). 289: 872–874.

Pocock,S., Shaper, A., Ashby, D., Delves, H., and Clayton B. 1988. The relationship between blood lead, blood pressure, stroke, and heart attacks in middle-aged British men. Environ Health Perspectives. 78: 23–30.

Popovac, D, Graziano, JH, Seaman, C, Kaul, B, Colakovic, B, Osmani, L, Haxhiu, M, Begraca, M, Bozovic, Z, and Mikic, M. 1982. Elevated blood lead in a population near a smelter in Kosovo, Yugoslavia. Archives of Environmental Health. 37: 19-23.

Polverini PJ. 1997. Role of the macrophage in angiogenesisdependent diseases. *EXS* 79: 11.

Pueschel, S.M., Kopito, L., Schwachman, H. 1972. Children with an increased lead burden. A screening and follow-up study. J. Am. Med. Assoc. 222, 462-466.

Rabinowitz M, Wetherill G, Kopple J. 1976. Kinetic analysis of lead metabolism in healthy humans. Journal of Clinical Investigations. 58: 260-270.

Rabinowitz M, Wetherill G, Kopple J. 1980. Effects of food intake and fasting on gastrointestinal lead absorption in humans. American Journal of Clinical Nutrition 33: 1784-88.

Ridker PM, Hennekens CH, Buring JE, Rifai N. 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843.

Rohde LE, Hennekens CH, Ridker PM. 1999. Cross-sectionalstudy of soluble intercellular adhesion molecule-1 and cardiovascular risk factors in apparently healthy men. Arterioscler Thromb Vasc Biol 19: 1595

Romeo, R., Aprea, C., Boccalon, P., Orsi, D., Porcelli, B., Sartorelli, P. 1996. Serum erythropoietin and blood lead concentrations. Int. Arch. Occup. Environ. Health 69, 73-75.

Rothenberg SJ, Kondrashov V, Manalo M, et al. 2002. Increases in hypertension and blood pressure during pregnancy with increased bone lead levels. American Journal of Epidemiology. 156:1079–87.

Rosen, J, Crocetti, A, Balb, K, Balbi, J, Baily, C, Clemente, I, Redkey, N, Grainger, S. 1993. Bone lead content assessed by L-line x-ray fluorescence in lead-exposed and non-lead-exposed suburban populations in the United States Proc. National Academy of Sciences. USA 1990.

Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809.

Safar ME, Levy BI, Struijker-Boudier H. 2003. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation 107:2864–2869

Sakata, S., Shimizu, S., Ogoshi, K., Hirai, K., Ohno, Y., Kishi, T., et al. 2007. Inverse relationship between serum erythropoietin and blood lead levels in Kathmandu tricycle taxi drivers. Int. Arch. Occup. Environ. Health 80, 342_345.

Schroeder HA, Tipton IH. 1968. The human body burden of lead. Arch Environ Health 17:965-978.

Schwartz, J. 1988. The relationship between blood lead and blood pressure in the NHANES II survey. Environ. Health Perspect 78, 15–22.

Schwartz, J. 1991. Lead, blood pressure, and cardiovascular disease in men and women. Environmental Health Perspectives 91: 71–75.

Schwartz J. 1995. Lead, blood pressure, and cardiovascular disease in men. Arch Environ Health 50:31–37.

Schwartz, B.S., Lee, B.-K., Lee, G.-S., Stewart, W.F., Simon, D., Kelsey, K., et al. 2000. Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with polymorphisms in the vitamin D receptor and δ-aminolevulinic acid dehydratase genes.Environ. Health Perspect. 108, 949-954.

Schütz, A., S. Skerfving, J. Ranstam, and J.-O. Christoffersson. 1987a. Kinetics of lead in blood after the end of occupational exposure. Scand. J. Work Environ. Health 13:221 231.

Scinicariello, F., Yesupriya, A., Chang, M.H., Fowler, B.A. 2010. Modification by ALAD of the association between blood lead and blood pressure in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. Environ. Health PerspectIVE 118 (2), 259–264.

Scinicariello, F., Abadin, H., Murray, H. 2011. Association of low-level blood lead and blood pressurein NHANES 1999–2006 Environmental Research 111; 1249–1257.

Selbst SM, Sokas RK, Henretig FM, Weller SC, Tershakovec AM. 1993. The effect of blood lead on blood pressure in children. Journal of Environ Pathol Toxicol Oncology. 12; (4) 213-8.

Selevan, S.G.; Landrigan, P.J. Stern, F.B.; Jones, J.H. 1985. Mortality of lead smelter workers. Am. J. Epidemiol. 122:673-683.

Sharp, D.S., J. Osterloh, C.E. Becker, B. Bernard, A.H. Smith, J.M. Fisher, S.L. Syme, B.L. Holman, and T. Johnston. 1988. Blood pressure and blood lead concentration in bus drivers. Environmental Health Perspectives. 78:131 137.

Smith, C. M.; Wang, X.; Hu, H.; Kelsey, K. T. 1995. A polymorphism in the δ-aminolevulinic acid dehydratase gene may modify the pharmacokinetics and toxicity of lead. Environ. Health Perspectives. 103: 248-253.

Silva PA., Hughes P., Williams S., Faed JM. 1988. Blood lead, intelligence, reading attainment, and behavior in eleven year old children in Dunedin, New Zealand. Journal of Child Psychology and Psychiatry. 1988; 29(1): 43-52.

Singer, S., and G. L. Nicolson. 1972. The fluid mosaic model of cell membranes. Science. 172:720–730.

Staessen, J. A.; Bulpitt, C. J.; Fagard, R.; Lauwerys, R. R.; Roels, H.; Thijs, L.; Amery, A. 1994. Hypertension caused by low-level lead exposure: myth or fact? J. Cardiovasc. Risk 1: 87-97.

Steck, T. L. 1974. The organization of proteins in the human red blood cell membrane. Journal of Cell Biol. 62:1-19.

Thomas LA. 1995. "The difficult quest of Herbert Needleman." National Wildlife, Vol. 33. # 3: 20 (6).

Todd AC, Chettle DR. 1994. In vivo X-ray fluorescence of lead in bone: review and current issues. Environ Health Perspect 102:172-177.

Tsaih, S.W.; Korrick, S.; Schwartz, J.; Amarasiriwardena, C.; Aro, A.; Sparrow, D.; Hu, H. 2004. Lead, diabetes, hypertension, and renal function: the Normative Aging Study. Environ. Health Perspect. 112, 1178-1182.

U.S. EPA. Environmental Protection Agency. 2006. Air Quality Criteria for Lead (Final).U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC, USA.

U.S. EPA. Integrated Science Assessment for Lead. 2012. EPA/600/R-10/075B.

U.S. Department of Energy. 1994. Office of Environmental Management, Risk assessment information system, Formal Toxicity Summary for Lead.

Vander AJ. Chronic effects of lead on the renin-angiotensin system. 1988. Environ Health Perspect.78:77-83.

Vaziri ND, Ding Y, Ni Z, and Gonick HC. 1997. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. Kidney Int 52:1042–1046.\

Vaziri, ND, Khan, M. 2007. Interplay of reactive oxygen species and nitric oxide in the pathogenesis of experimental lead-induced hypertension. Clin Exp Pharmacol Physiol 34: 920–925.

Vupputuri, S.; He, J.; Muntner, P.; Bazzano, L.A.; Whelton, P.K.; Batuman, V. 2003. Blood lead level is associated with elevated blood pressure in blacks. Hypertension. 41, 463-468.

Victery, CR. Miller and B.A. Fowler. 1984. Lead accumulation by rat renal brush border membrane vesicles. J. Pharmacol. Exp. Ther., 231 589.

Waldron, H.A. 1966. The anemia of lead poisoning: A review. Br. J. Ind. Med. 23:83 100.

Warren MJ, Cooper JB, Wood SP, Shoolingin-Jordan PM. 1998. Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. Trends in Biochemical Sciences. 23: 217–21.

Wasserman, G, Graziano, J, Factor-Litvak, P, Popovac, D, Morina, N, Musabegovic, A, Vrenezi, N, Capuni-Paracka, S, Lekic, V, Preteni-Redjepi, E. 1992. Independent effects of lead exposure and iron deficiency anemia on developmental outcomes at age 2 years. Journal of Pediatrics 121: 695-703.

Wasserman, G, Graziano, JH, Factor-Litvak, P, Popovac, D, Morina, N, Musabegovic, A, Vrenezi, N, Capuni-Paracka, S, Lekic, V, Preteni-Redjepi, E, Hadjialjevic, S, Slavkovich, V, Kline, J, Shrout, P, Stein, Z. 1994. Consequences of lead exposure and iron supplementation on childhood development at age 4 years. Neurotoxicology and Teratology 16: 233-240.

Wasserman, G, Liu, X, Lolacono, N, Factor-Litvak, P, Kline, J, Popovac, D, Morina, N, Musabegovic, A, Vrenezi, N, Capuni-Paracka, S, Lekic, V, Preteni-Redjepi, E, Hadzialjevic, S, Slavkovich, V, and Graziano, J. 1997. Lead Exposure and Intelligence in 7-Year-old Children: The Yugoslavia Prospective Study. Environmental Health Perspectives 105, 9, 956-962.

Wasserman, G, Staghezza-Jaramillo, B, Shrout, P, Popovac, D, Graziano, JH. 1998. The effect of lead exposure on behavior problems in pre-school children. American Journal Public Health 88: 481-486.

Wassermann, G, Musabegovic, Liu, X, Kline, J, Factor-Litvak, P, Graziano, J. 2000. Lead exposure and motor functioning in 4 ½ year-old children: The Yugoslavia prospective study. Journal of Pediatrics 137:4-555-561.

Wasserman, G, Factor-Litvak, P, Liu, X, Todd, A, Kline, J, Slavkovich, V, Popovac, D, Graziano, JH. 2003. The relationship between blood lead, bone lead and child intelligence. Child Neuropsychology 9: 22-34.

Watson ML, Workman RJ, Herzer W, et al. 1986.Systematic synthesis of prostaglandin I_2 following sustained infusion of angiotensin II in conscious dogs. European Journal of Pharmacology 127(1-2):9-16.

Weaver VM, Lee BK, Ahn KD, Lee GS, Todd AC, Stewart WF, Wen J, Simon DJ, Parsons PJ, Schwartz BS. 2003. Associations of lead biomarkers with renal function in Korean lead workers. Occup Environ Med. 60:551–562.

Weaver, V.M.; Lee, B.K.; Todd, A.C.; Ahn, K.D.; Shi, W.; Jaar, B.G.; Kelsey, K.T.; Lustberg, M.E.; Silbergeld, E.K.; Parsons, P.J.; Wen, J; Schwartz, B.S. 2006. Effect modification by delta-aminolevulinic acid dehydratase, vitamin D receptor, and nitric oxide synthase gene polymorphisms on associations between patella lead and renal function in lead workers. Environ. Res. 102, 61-69.

Weaver, V., Ellis, L., Lee, BK.,Todd,A., Shi,W., Ahn, K., Schwartz, B. 2008. Associations Between Patella Lead and Blood Pressure in Lead Workers DOI10.1002/ajim.20573. Wiley InterScience (www.interscience.wiley.com)

Weaver, VM, Griswold, M, Todd, AC, Jaar, BG, Ahnd, K-D, Thompson, CB, Lee, B-K. 2009. Longitudinal associations between lead dose and renal function in lead workers. Environ Res. 109:101-107.

Weeden RP, D'Haese P, Van de Vyver FL, et al. 1986. Lead nephropathy. Am J Kidney Dis 3(5): 380-3.

Weiss, S.T.; Muñoz, A.; Stein, A.; Sparrow, D.; Speizer, F.E. 1986. The relationship of blood lead to blood pressure in a longitudinal study of working men. Am. J. Epidemiol.123:800-808. (cited in EPA, 1986e).

White, J.M., Harvey, D.R. 1972. Defective synthesis of α and β globin chains in lead poisoning. Nature; 236, 71_73.

WHO Regional Office for Europe, Copenhagen, Denmark. 2001. Chapter 6.7 Lead Air Quality Guidelines - Second Edition Wu, T., ' Shen, C., Ko, K., Guu, C., Gao, H., Lai, J., Chen C., Chng, P. (1996). Occupational lead exposure and blood pressure. International Journal of Epidemiology. 25, No. 4.

Yu CC, Lin JL, Lin-Tan DT. 2004. Environmental exposure to lead and progression of chronic renal diseases: a four-year prospective longitudinal study. J Am Soc Nephrol. 15:1016–1022.

Ziegler EE, Edwards BB, Jensen RL, Mahaffey KR and Fomon SJ. 1978. Absorption and retention of lead by infants. Pediatric Research, 12, 29-34.

Zhang, LF; Peng, SQ; Wang, S. 2009a. Decreased aortic contractile reaction to 5-hydroxytryptamine in rats with long-term hypertension induced by lead (Pb2+) exposure. Toxicol Lett 186: 78-83.

Zhang, A; Hu, H; Sánchez, BN; Ettinger, AS; Park, SK; Cantonwine, D; Schnaas, L; Wright, RO; Lamadrid-Figueroa, H; Tellez-Rojo, MM. 2012. Association between Prenatal Lead Exposure and Blood Pressure in Female Offspring. Environ Health Perspectives 120 (3) 445-450.

CHAPTER IV: Long-Term Effects of Environmental Lead exposure on Blood Pressure and Plasma Soluble Cell Adhesion Molecules in Young Adults: A Follow-up Study of a Prospective Cohort in Kosovo

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Abstract

BACKGROUND: Epidemiologic studies examining the relationship between environmental lead (Pb) exposure and blood pressure (BP) generally report small associations between blood lead (BPb) and BP. However, these studies are predominantly cross-sectional. Currently no data evaluate whether Pb exposure in early life is associated with BP later in life. In addition, no epidemiologic studies evaluate associations between either current or past Pb exposure and serum levels of markers of systemic inflammation and endothelial dysfunction, including soluble intercellular adhesion molecule (sICAM-1) and soluble vascular cell adhesion molecule (sVCAM-1). Systemic inflammation and endothelial dysfunction have been suggested as mechanisms for the Pb-BP relationship. Here we evaluate these relationships prospectively.

OBJECTIVE: We investigated the association between prenatal, early childhood, and concurrent Pb exposure and BP and serum levels of circulating sICAM-1 and sVCAM-1 later in life.

METHODS: From our original prospective birth cohort study in Mitrovica (a mining town) and Prishtina, Kosovo, from 1985-1998, we located and assessed BPb and BP in 101 participants (mean age 24.9 years). We first examined the association between concurrent BPb and BP. We then examined associations between BP and a series of cumulative lifetime exposure measures from the prenatal period onward. Lastly, we examined the association between concurrent BPb levels and circulation levels sICAM-1 and sVCAM-1.

RESULTS: BPb levels, measured every six months from birth through age 12, varied across the study sample especially in early life. At age 25, the mean BPb was 4.91 µg/dl (range = 1.41-16.4 µg/dl) in Mitrovica and 1.68 µg/dl (range = 0.69-3.51 µg/dl) in Prishtina. We found small

adjusted associations between BPb measured in childhood and at age 25 on systolic BP (sBP) and diastolic BP (dBP). For example, after adjustment, the estimated regression coefficient relating current BPb to sBP was 1.04 (95% confidence interval (CI) 0.09, 1.86) mm Hg per log unit increase in BPb and the estimated regression coefficient relating current BPb to dBP was 0.35 (95% CI -0.13, 0.83) mm Hg per log unit increase in BPb. We also found small, albeit not statistically significant, associations with sICAM and sVCAM. In addition, we did not see evidence of sICAM-1 and sVCAM-1 mediating the relationship between BPb and BP.

CONCLUSION: Current study results, along with previously reported findings on this cohort, provide evidence for the hypothesis that exposure to Pb either prenatally or in early adulthood, may lead to increased BP and increased circulating levels of sICAM-1 and sVCAM-1 later in life.

KEY WORDS: Blood lead, systolic/diastolic blood pressure, vascular inflammation, Kosovo, environmental lead exposure.

Background

Public health initiatives have been successful in dramatically reducing exposure to environmental lead (Pb), especially in the United States (Lustberg et al., 2002; Muntner et al., 2005). However, even at low-levels of Pb exposure, there is support for an association between Pb and cardiovascular health and all-cause mortality among US population (Pirkle et al., 1994; Menke et al., 2006). A significant body of research has reported associations between blood Pb concentrations (BPb) and blood pressure (BP) in populations with BPb at levels that had until recently been considered as "safe" (i.e., < 10 µg/dl) (Navas-Ancien et al., 2007, Nash et al, 2003; Vupputuri et al. 2003; Glenn et al., 2003). Support for a relationship between Pb and BP comes from a wide range of animal studies as well as clinical and epidemiological studies of Pb-exposed workers and the general population. A significant number of reviews and meta-analyses based on more than 30 studies and more than 50,000 participants have reached the prevailing conclusion that there is significant association between BPb and BP [Sharp et al., 1987; Hertz-Picciotto and Croft, 1993; Schwartz, et al., 1995; Staessen et al., 1994, 1996; Nawrot et al., 2002; U.S. Environmental Protection Agency (U.S. EPA) 2006], Scinicariello et al., 2010].

In addition, a limited number of published studies examining associations between Pb exposure and BP in children have reported contrasting results. No association was observed between BPb and BP in children (Selbst et al., 1993). Increasing *cord* BPb level was associated with significantly higher baseline sBP and marginally higher baseline dBP at 9.5 years of age (Gump et al., 2005). Maternal bone-Pb (tibia-Pb) was associated with increase in sBP and dBP only in girls (Zhang et al., 2012). Analyses of the BPb and BP relationship at age 5.5 in the Yugoslavia study of lead exposure, pregnancy outcomes and child development, found small,

non-statistically significant, associations between BPb and both sBP and dBP (Factor-Litvak, et al 1996). Although modest, there is some evidence that prenatal Pb exposure may be associated with BP later in life. Here, we extend these findings by evaluating the associations between prenatal and childhood lead exposure and BP in early adulthood.

We also examine associations between BPb and two markers of inflammation and endothelial dysfunction, sICAM-1 and sVAM-1, both of which are proposed as mechanisms for the Pb-BP associations. Both markers are associated with CVD risk factors such as hypertension, smoking and frequent alcohol consumption (Blann et al., 1997; Rohde et al., 1999) and are related to increasing sBP (Chae et al., 2001). Although Pb and arsenic (As) are not very similar (one is a heavy metal and the other a metalloid), As is usually grouped with metals in terms of its toxicology. Both are associated with outcomes in multiple systems and both and have similar molecular mechanisms, as they interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups). Also, like Pb, As crosses the placental barrier, can affect the hematopoietic system, cardiovascular system, and there is accumulating evidence of damage to central nervous system (ATSDR, 2004). Previous studies found a positive relationship between chronic As exposure and sICAM-1 and sVCAM-1 (Chen et al., 2007).

Methods

a) Study Design

The original cohort has been previously described (Factor-Litvak et al., 1999). Briefly, pregnant women were recruited between 1984 and 1985 in two towns, Mitrovica, the site of the Trepça mines, smelter and battery plant and Prishtina, the capitol which was relatively

unexposed. Offspring were followed frequently for BPb measures, neurocognitive development and physical examinations until age 12.5.

We located and identified 101 members of the original cohort from the Yugoslavia study of environmental lead, pregnancy outcomes and child development and requested their participation in a follow-up study, in which each participant would be evaluated once to assess their current BPb, BP and serum sICAM-1 and sVCAM-1. Subjects were recruited through television, radio and newspaper advertising and through "word of mouth". Following a procedure to insure that they were from the original cohort, we set up appointments for all participants to report to a central location in each town in order conduct interviews, fill-out questionnaires, and collect biological samples. All questionnaires were administered in the participant's primary language, Albanian or Serbian. While we contacted and recruited study participants in both towns, we encountered more challenges finding and recruiting the participants from Prishtina. This may be due to the fact that the city expanded greatly in the past 8-10 years with a number of changes especially in the ethnic makeup of the population. In addition, once contacted, those in Prishtina were less interested, and more of them refused to take part in the study. This may be due to the fact that they did not view the study as beneficial to them as did those in Mitrovica. Another factor that may have influenced the recruitment may have been that the overwhelming majority of the pre-war Serbian population in Prishtina had been uprooted from Kosovo at the beginning of NATO campaign in 1999. However, the Serbian population in Mitrovica (Northern part of the city) for the most part has remained in place. There may have been some temporary migration during the few months at the height of the war, but it has been reported that most had returned to their homes shortly after. Similarly, the Albanian population in Mitrovica has remained very much intact with similar pattern of

temporary migration during the few months (1-3 months) of the height of the hostilities. Their temporary migration was mostly to camps in neighboring Albania and Montenegro. However, as the hostilities diminished, the overwhelming majority returned to their homes.

b) Data Collection and Laboratory Analysis

Demographic information and other lifestyle characteristics such as smoking, were collected via questionnaire. We accessed existing data from the mid-pregnancy, delivery, and childhood questionnaires to ascertain the life-style characteristics of the mothers of cohort members as needed. Data on the outcomes were collected as follows:

i) BP was measured using an automated monitor three times (at 1-minute intervals) at the completion of the interview and questionnaire but before the blood draw (Omron BP Monitor, Model # 785, Lake Forest, Illinois). For the statistical analysis, we used the standard research method of averaging the two last measures. As anticipated, the first BP measure was higher than subsequent measures; the mean difference between the first and third BP measures was 2.53 mmHg (p< 0.001) and 2.33 (p< 0.001) for sBP and dBP respectively. This is likely due to the "white coat" effect (Mancia et al., 1983; Pickering et al., 1988).

ii) Blood samples were collected in EDTA vacutainers by venipuncture from each participant. BPb levels were analyzed using a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), model AAnalyst 600 (Perkin-Elmer, Shelton, CT) using a method modified after Fernandez and Hilligoss (1982). Q Columbia University's laboratory participates

in the Center for Disease Control and Prevention quality control program for BPb; in the past three years, the interclass correlation between the expected and observed BPb was 0.99.

iii) Human sICAM-1 and sVCAM-1:   Serum levels sICAM-1 and sVCAM-1 were analyzed utilizing the Quantikine Human sICAM-1/CD54 and sVCAM-1 kits that included pre-coated microplates, standard, calibrator diluent, wash buffer, color reagents, and "stop" solution (R&D Systems, Minneapolis, MN).   "Inter-precision coefficients of variation for sVCAM-1 were 4.6% for quality control samples, and 5.7% for study samples. The corresponding values for sICAM-1 were 2.8% and 2.5%."

c) Statistical Analysis

We first conducted descriptive analyses to compare the distributions of variables of interest, by utilizing t-test or chi-square tests, and unadjusted bivariate linear regression models relating exposure to outcomes. Linear regression was used to model associations between BPb and BP, sICAM-1and sVCAM-1 controlling for ethnicity, gender, body mass index (BMI), smoking, employment status, and education. Potential confounders were retained in the final models if the estimated coefficient relating PBb to BP, sICAM-1 and sVCAM-1 changed at least 10% with their inclusion. In addition, we also assessed for possible mediating effects of sICAM-1 and sVCAM-1; a reduction in the estimated regression coefficient relating BPb to BP with sICAM-1 and/or sVCAM-1 in the model was regarded as evidence of mediation.

BPb measures were available for each subject from birth through 12.5 years of age, and at the follow up. We first examined the associations with BPb measured at the time of follow up. Second, we calculated cumulative lead exposure, using the trapezoidal area under the curve for

various age periods. We calculated the area under the curve for exposure between ages 0 and 2, ages 2 and 4, ages 4 and 7 and ages 7 to 12. Third, we also examined the associations between BP and the total area under the BPb vs. age curve (AUC). All analyses were performed using SAS version 9.3 (SAS Institute, Carey, NC).

Results

As shown in **Table 4-7**, the study participants were more likely to reside in Mitrovica and were more likely to be Albanian. Compared to the original study cohort, the current cohort is predominantly Albanian, especially in Prishtina. Over 80% of the follow up sample were from Mitrovica compared to 55% in the original birth cohort. Of the 101 participants in this study, those residing in Prishtina were better educated and more likely to be employed. Smoking was more prevalent in Prishtina (66% vs 29% in Mitrovica). The follow up sample gender distribution reflected that of the original cohort. Biomarkers and anthropometric characteristics are also listed in **Table 4-7.** Mean BPb concentrations remained significantly higher in Mitrovica residents than in those from Prishtina, even though the lead smelting plant drastically diminished its operations in late 1990's and closed at the onset of the war in 1999. The smelting operations have remained closed and only ore mining operations at reduced capacities have resumed in the last few years. Average BPb concentrations between birth and age 12.5 and at age 25 are illustrated in **Figure 4-8**. At age 12.5, the mean BPb concentrations were 30.6 µg/dl (SD=8.8 µg/dl) in Mitrovica, and 6.1 µg/dl (SD=1.6 µg/dl) in Prishtina. Current BPb levels ranged from 1.41-16.4 µg/dl and 0.69-3.51 µg/dl in Mitrovica and Prishtina respectively.

Mean sBP were 129.90 (SD=14.87) mmHg and 125.64 (SD=9.45) mmHg in Mitrovica and Prishtina, respectively **(Table 4-8)**. Mean dBP were 81.31 (SD=7.51) and 79.00 (SD=5.09)

in Mitrovica and Prishtina, respectively. Higher sBP and dBP was found among Serbians, males, those with higher body mass index (BMI), those with lower education, and non-smokers.

Results from the regression models relating BPb to sBP and dBP are shown in **Table 4-9**. Only smoking and BMI met the criteria for potential confounding. The unadjusted regression coefficient relating BPb to sBP was 1.47 (95 % CI 0.64, 2.29), corresponding to a 1.47 mmHg increase in sBP for each log unit increase in BPb. After adjusting for potentially confounding variables, the magnitude of the regression coefficient diminished to 1.04 mmHg (95 % CL 0.09, 1.86) increase in sBP per log unit increase in BPb. This relationship is illustrated in Figure 9. The unadjusted regression coefficient relating BPb to dBP was 0.57 mmHg (95 % CI 0.14-1.00) for each log unit increase in BPb. After adjustment, the magnitude of the regression coefficient decreased to 0.33 mmHg (95 % CI -0.13, 0.83).

We also examined the associations between sBP and the total area under the BPb vs. age curve (AUC) for post-natal periods of birth to age 2, 2-4, 4-7, and 7-12 years (data not shown). These measures of BPb were not associated with either sBP or dBP. However, they were in the anticipated direction.

We found small, although not statistically significant, associations between BPb and both sICAM-1 and sVCAM-1. This association is illustrated in **Figure 4-10**. The unadjusted regression coefficient sICAM-1 was 4.76 ng/ml (95 % CI 1.14, 8.38) for each unit increase in BPb (p< 0.01). After adjusting for BMI, ethnicity, and smoking, the magnitude of the regression coefficient diminished to 3.37 ng/ml (95 % CL -0.42, 7.19) for each unit increase in BPb. The unadjusted regression coefficient for sVCAM-1 was 12.92 ng/ml (95 % CL -1.32, 27.17) for each log unit increase in BPb). After adjustment, the regression coefficient declined to 11.56

ng/ml (95 % CL -4.49, 27.33) for each log unit increase in BPb **(Table 4-10)**. This relationship is also illustrated in **Figure 4-11**.

We also examined the associations between sICAM-1 and sVCAM-1 and the total area under the BPb vs. age curve (AUC) for post-natal periods of birth to age 2, 2-4, 4-7, and 7-12 years (data not shown). These measures of BPb were not associated with either sICAM-1 or sVCAM-1.

Discussion

We tested the hypothesis that higher levels of prenatal and early life Pb exposure are associated with an increase in BP later in life. Lead exposure was estimated using available past measurements of BPb from birth through age of 12, as well as concurrent BPb at 25 years of age. Between ages 12 and 25 years, the mean BPb fell from 29.7 to 4.91 µg/dl in Mitrovica, and from 5.73 to 1.67 µg/dl in Prishtina, most probably due to the demise of the lead industry in the area. The declines in BPb correspond to an apparent half-life of elimination of 4.9 and 7.4 years in Mitrovica and Prishtina, respectively **(Appendix Table 14)**. We examined the associations between BP and the total area under the BPb vs. age curve (AUC) as well as the AUC for early post-natal periods (birth to age 2y, 2-4y, 4-7y, and 7-12y). While we found positive association between concurrent BPb and sBP and dBP, earlier measures of BPb were not associated with either BP measure. In addition, the association between BPb and circulating levels of two markers of endothelial cell dysfunction, sICAM-1 and sVCAM-1, suggested an adverse association; however sample size limitations resulted in confidence limits for the estimated association straddling the null.

Accumulating scientific evidence suggests that exposure to Pb may result in increased BP across a wide range of populations. In its review of many epidemiological and occupational studies, the U.S. Environmental Protection Agency concluded that every doubling of BPb levels is associated with a ~1.0 mmHg increase in sBP and ~0.6 mmHg increase in dBP (EPA, 2006), magnitudes close to those found in the present study. Early studies, including three large population-based studies [the British Regional Heart Study (BRHS) (Pockock et al., 1988), the National Health and Nutrition Examination Survey (NHANES II and III) (Harlan et al., 1988; Den Hond et al., 2002, Nash et al., 2003) and Welsh Heart Programme study (Elwood et al., 1988)] observed associations between BPb levels and increased BP. Several meta-analyses reported similar relationships between BPb and BP [Sharp et al. 1987; Hertz-Picciotto and Croft, 1993; Nawrot et al. 2002; Schwartz, et al., 1995; Staessen et al., 1994, 1995; U.S. EPA 2006]. In a meta-analysis of 23 studies, a doubling of BPb from 5 to 10 µg/dl was associated with increases in systolic blood pressure (sBP) and diastolic blood pressure (dBP) of 1.0 and 0.6 mmHg respectively) (Staessen et al., 1994). Another meta-analysis comprised of 15 publications reported an average of 1.25 mmHg of sBP increase for every doubling of BPb concentration (Schwartz, et al., 1995). Our findings add to a number of earlier cross-sectional analyses that reported increases in sBP and dBP in relation to BPb across various populations (Pocock et al., 1988; Pirkle et al., 1985; Harlan et al., 1988; Landis et al., 1988;) including pregnant women (Factor-Litvak et al., 1993).

An association between bone Pb and BP has also been reported. Bone Pb levels had a stronger association to BP and hypertension (defined as repeated measurements of > 140/90 mmHg) than to BPb level in adult men (Cheng et al., 2001; Hu et al., 1996). These studies,

although larger had a number of limitations, notably that both Pb exposure and BP were measured at only one point in time.

Studies examining associations between Pb exposure and BP in children have reported differing results. Among members of this study's cohort at 5.5 years of age a 10 μg/dl increase in BPb was associated with a small increase of 0.5 (95% CL=-0.2, 1.3) mmHg) for sBP, and a 0.4 (95%, CL=-0.1, 0.9) mmHg increase in dBP (Factor-Litvak et al., 1996). No association was observed between increased BPb levels and BP in a cohort of 149 children in Philadelphia (1-10 years old) (Selbst et al., 1993). Another study reported that increasing *cord* BPb levels were associated with significantly higher baseline sBP and marginally higher baseline dBP in children at 9.5 years of age, while no association between BP and *postnatal* BPb levels were found (Gump et al., 2005). A clinical study of 780 children also found no association between BPb and BP (Chen et al., 2006). More recently, Zhang et al (2012) found that *maternal* bone-Pb (tibia-Pb) was associated with increase in sBP and dBP only in girls and no associations were found between cord and *postnatal* (early childhood) BPb levels and BP.

The biological plausibility of the BPb and BP relationship has been documented in a number of animal studies. In rats, exposure to Pb for 4-5 months (leading to BPb concentration of 30-40 μg/dL) was reported to induce hypertension (Vander et al. 1988; Novak, et al. 1993). More recently, sBP increased in rats after exposure to 90-10,000 ppm Pb (as Pb-acetate in drinking water) for various time periods that resulted in BPb levels between 19.3-240 μg/dL (Mohammad et al., 2010). The disruption of the biological functions that can interfere with tightly regulated processes such as cell signaling, intracellular ion homeostasis, ion transport, energy metabolism, and enzyme functions are some of possible ways of Pb-induced cardiovascular toxicity. One plausible mechanism concerns the adverse effects of Pb on the

kidney renin-angiotensin system (Vander et al. 1988). In addition, Pb may affect sites of the cardiovascular system that control heart excitability and contractility, or may impact compartments of the central nervous system that regulate BP and other cardiovascular functions (Kopp et al., 1988; Navas-Ancien et al., 2007). Pb-induced oxidative stress via generation of reactive oxygen species (ROS) has been thought to be a primary contributory factor in the pathogenesis of its adverse health effects (Vaziri & Khan et al., 2007). A number of studies have demonstrated a role for oxidative stress in the pathogenesis of Pb-induced hypertension, mediated by the inactivation of nitric oxide (•NO) and down-regulation of soluble guanylate cyclase (sGC) (Dursun et al., 2005; Vaziri et al., 1999).   The reduction of the vasodilator •NO can lead to increased vasoconstriction and subsequently BP.

It has also been reported that Pb may be an important factor of stimulation and proliferation of vascular smooth muscle cells (Fujivara et al., 1995). Recent studies indicate that the concentrations of the circulating sICAM-1 and sVCAM-1 appear to have predictive value for the identification of early atherosclerotic lesions and future cardiovascular disease (CVD) (Krauss et al., 2002; Blankenberg et al., 2001; Ridker et al., 2000; Hwang et al., 1997). These adhesion molecules play a crucial role in the immune system response by promoting cell–cell and cell–stroma interactions and leukocyte migration (Carlos & Harlan, 1994). The process of adhesion of the leukocytes to the endothelial cells and subsequent trans-endothelial migration is an important step in the atherosclerosis, arthritis, and cancers (Ross, 1993; Polverini, 1997). While studies have shown that age is the most powerful independent predictor of the increasing levels of sICAM-1 and sVCAM-1, the effects of Pb may also play a significant role in this process (Morisaki et al., 1997). Elevated sICAM-1 levels have been associated with CVD risk factors such as hypertension, smoking and frequent alcohol consumption (Blann et al., 1997;

Rohde et al., 1999) and were found to be related to increasing sBP (Chae et al., 2001). Angiotensin II, which is a potent vasoconstrictor, stimulates the sICAM-1 expression and as such, it may play a role in the increase in BP (Mervaala et al., 1999). Our study found a modest association between BPb levels sICAM-1 and sVCAM-1. This finding along with the reported slight increases in the sBP, may be early signs indicating negative effects of Pb on cardiovascular system. However, we did not find sICAM-1 or sVCAM-1 to be a mediator in the relationship between BPb and sBP (data not shown).

In the previous study of this cohort at age 5.5 years old (Factor-Litvak et al., 1996), a slight increase in sBP pressure was reported in those with high BPb levels. Although, the current cohort is smaller (N=101) than the previous one (N=282), the data analysis showed that the study sample of the Pb-exposed town was representative of the larger 1996 study sample (data not shown).

We were able to control for potential confounding variables and examined BMI, gender, ethnicity, education, and smoking. However, only BMI and smoking met the criteria for potential confounding. Interestingly, some findings (Berglund et al., 1975; Hughes et al., 1991; Charlton et al., 1995) suggest that casual smoking may reduce BP, although most studies link smoking to increased BP (Tuomilehto et al., 1982; Mann et al., 1991). There have been suggestions that smokers may refrain from smoking before medical examinations, which could possibly cause a sharp short-lived drop in BP (Havlick et al. 1980). Also, validation and standardization of the self-reported smoking habits which can vary significantly from person to person need to be taken into account (Charlton, 1995).

This study has a number of strengths. First, there was a wide range of BPbs measured early in life, albeit less of a range at age 25, making possible the analysis of early life exposure and

later outcomes. Second, the sample has few adult risk factors for high BP, i.e. over 70% had BMIs between 20 and 24.9 and most were "light" smokers   (smoking a few to < 20 cigarettes a day). The study is limited in that we only followed a select 20% of the sample; however, there is no reason to assume that the biological relationships would differ between those followed and those not followed. A second limitation pertains to the single measure of CVD risk markers. Another limitation is the 12 ½ year gap in the BPb measurements. Finally, our analysis is based on a select population residing in Kosovo and the results may not be generalizable to other populations

In summary, we found a statistically significant association between BPb measured concurrently and sBP, and a marginally significant association between BPb and dBP, which is consistent with previous studies. Furthermore, our data suggest an association between concurrent BPb and levels of circulating sICAM-1 and sVCAM-1, possibly a mechanism by which Pb may lead to increased BP. The findings support the hypothesis that the exposure to Pb poses a risk for elevated BP.

REFERENCES (Chapter IV)

Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Interaction profile for arsenic, cadmium, chromium and lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service

Berglund G, Wilhelmsen L. 1975. Factors related to blood pressure in a general population sample of Swedish men. Acta Med Scand. 198:291–298.

Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, et al. (2001). Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 104:1336–1342.

Blann, A.D., Nadar, S.K. and Lip, G.Y. 2003. The adhesion molecule P-selectin and cardiovascular disease. Eur. Heart J., 24(24):2166–79.

Carlos, T. M. and Harlan, J. M. 1994. Leukocyte-endothelial adhesionmolecules. Blood 84, 2068-2101.

Chae CU, Lee RT, Rifai N, Ridker PM. 2001. Blood pressure and inflammation in apparently healthy men. Hypertension. 38(3):399-403.

Chen, Y; Santella, R; Kibriya M; Wang, Q; Kappil, M; Verret, M; Graziano, J; Ahsan H. 2007. Association between Arsenic Exposure from Drinking Water and Plasma Levels of Soluble Cell Adhesion Molecules. Environmental Health Perspectives; 115, 10, 1415-1420.

Chen, A; Rhoads,G; Cai, B; Salganik, M; Rogan, W. 2006. The Effect of Chelation on Blood Pressure in Lead-Exposed Children: A Randomized Study. Environmental Health Perspectives 114, 4, 579-583.

Cheng Y, Schwartz J, Sparrow D, Aro A, Weiss ST, Hu H. 2001. Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension: The normotensive aging study. Am. J. Epidemiol.; 153: 164–71.

Charlton, A, While, D. 1995. Blood pressure and smoking: observations on a national cohort Archives of Disease in Childhood. 73: 294-297.

Den Hond, E.; Nawrot, T.; Staessen, J. A. 2002. The relationship between blood pressure and blood lead in NHANES III. J. Hum. Hypertens. 16: 563-568.

Dursun, N; Arifoglu, C; Suer, C; Keskinol, L. 2005. Blood pressure relationship to nitric oxide, lipid peroxidation, renal function, and renal blood flow in rats exposed to low lead levels. Biol Trace Elem Res 104: 141-149.

Elwood, P.C. Yarnell, J.W.G.; Oldham, P.D.; et al. 1988b. Blood pressure and blood lead in surveys in Wales. Am. J. Epidemiol. 127:942-945.

Factor-Litvak, P, Graziano, JH, Stein, Z. 1993. Increased risk of proteinuria among a cohort of lead-exposed pregnant women. Environmental Health Perspectives 101: 418-421.

Factor-Litvak, P, Kline, J, Popovac, D, Hadzialjevic, S, Lekic, V, Preteni-Redjepi, E, Capuni-Paracka, S, Slavkovich, V, Graziano, JH. 1996. Blood lead and blood pressure in young children. Epidemiology. 7: 633-637.

Factor-Litvak, P, Wasserman, G, Kline, J, Graziano, JH. 1999. The Yugoslavia prospective study of environmental lead exposure. Environmental Health Perspectives. 107: 9-15.

Fernandez, F, Hilligoss. 1982. An improved graphite furnace method for the determination of lead in bloos using matrix modification and L'vov platform. Atomic Spectroscophotometry. 3, 130-131.

Fujivara,C, Kaji, T, Yamamoto, C, Sakamoto, M, Kozuka, H. 1995. Stimulatory effect of lead effect of lead on the proliferation of cultured vascular smooth-muscle cells. Toxicology 98: 105-110.

Glenn, BS, Stewart, WF, Links, JM, Todd, AC, Schwartz, BS. 2003. The Longitudinal Association of Lead with Blood Pressure. Epidemiology; Vol. 14.

Gump, BB; Stewart, P; Reihman, J; Lonky, E; Darvill, T; Matthews, KA; Parsons, PJ. 2005. Prenatal and early childhood blood lead levels and cardiovascular functioning in 9 1/2 year old children. Neurotoxicol Teratol 27: 655-665.

Harlan, W. 1988. The relationship of blood lead levels to blood pressure in the US population. Environmental Health Perspectives 78: 9–13.

Havlik RJ, Garrison RJ, Feinleib M, et al. 1980. Evidence for additional blood pressure correlates in adults 20-56 years old. Circulation. 61: 710-5.

Hertz-Picciotto I, Croft J. 1993. Review of the relation between blood lead and blood pressure. Epidemiology Rev. 15: 352–73.

Hu H, Aro A, Payton M, et al. 1996. The relationship of bone and blood lead to of bone and blood lead to hypertension. The Normative Aging Study. JAMA 275:1171-6.

Hughes, K, Leong, W. P, Sothy, S. P. Lun, K. C and Yeo, P. P. B. 1991. Relationships between cigarette smoking, blood pressure and serum lipids in the Singapore general population. International Journal of Epidemiology, vol. 22, no. 4, pp. 637–643.

Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, GottoAM Jr, et al. 1997. Circulating adhesion molecules VCAM-1,ICAM-1, and E-selectin in carotid

atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 96:4219–4225.

Kopp SJ, Barron JT, Tow JP. 1988. Cardiovascular actions of lead and relationship to hypertension: a review. Environ Health Perspect. 78:91-99.

Krauss, T, Emons, G, Kuhn, W, Augustin, H. 2002. Predictive value of routine circulating soluble endothelial cell adhesion molecule measurements during pregnancy. Clinical Chemistry 48: 9, 1418–1425.

Landis, J.R, Flegal, K.M. 1988. A generalized Mantel-Haenszel analysis of the regression of blood pressure on blood lead using NHANES II data. Environ.

Lustberg M, Silbergeld E. Blood lead levels and mortality. Arch Intern Med. 2002. 162:2443-2449.

Mancia G, Grassi G, Pomidossi G, Gregorini L, Bertineri G, Parati G, et al. 1983.Effects of blood pressure measurement by the doctor on patient's blood pressure and heart rate. Lancet. 2: 695–698.

Mann SJ, James GD, Wang RS, Pickering TG. 1991. Elevation of ambulatory systolic blood pressure in hypertensive smokers: a case-control study. JAMA. 265:2226 –2228.

Menke A, Muntner P, Batuman V, Silbergeld EK, Guallar E. 2006. Blood lead below 0.48 {micro}mol/L (10 {micro}g/dL) and mortality among US adults. Circulation. 114:1388–1394.

Mervaala EM, Müller DN, Park JK, Schmidt F, Löhn M, Breu V, Dragun D, Ganten D, Haller H, Luft FC. (1999). Monocyte infiltration and adhesion molecules in a rat model of high human renin hypertension. Hypertension 33: 389.

Morisaki, N. 1997. New indices of ischemic heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hyper-cholesterolemia and ischemic heart disease. Atherosclerosis 131: 1, 43-48.

Mohammad, A; Ali, N; Reza, B; Ali, K. 2010. Effect of ascorbic acid supplementation on nitric oxide metabolites and systolic blood pressure in rats exposed to lead. Indian J Pharmacol 42: 78-81.

Munter, P, Menke, A, DeSalvo K, Rabito, F, Batuman, V. 2005. Continued decline of blood lead levels among adults in the US: The National Health and Nutrition Examination Survey. Archives of Internal Medicine. 165: 2155-2161.

Nash, D.; Magder, L.S.; Sherwin, R.; Rubin, R.J.; Silbergeld, E.K. 2003 Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women. JAMA, 289, 1523-1532.

Navas-Acien, A, Guallar, E, Silbergeld, E, Rothenberg, S. 2007. Lead Exposure and Cardiovascular Disease—A Systematic Review. Environmental Health Perspectives 115: 3, 472-82.

Nawrot TS, L Thijs, EM Den Hond, HA Roels and JA Staessen. 2002. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis Journal of Human Hypertension. 16, 123–131.

Novack R, Wiecek A, Exner B, Gretz N, Ritz E. 1993. Chronic lead exposure in rats: effects on blood pressure. Eur J Clin Invest. 23:433-443.

Ridker PM, Hennekens CH, Buring JE, Rifai N. 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843.

Rohde LE, Hennekens CH, Ridker PM. 1999. Cross-sectionalstudy of soluble intercellular adhesion molecule-1 and cardiovascular risk factors in apparently healthy men. Arterioscler Thromb Vasc Biol 19: 1595.

Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809.

Pickering TG, James GD, Boddie C, Harshfield GA, Blank S, Laragh JH. 1988. How common is white coat hypertension? JAMA. 259:225–228.

Pirkle, J, Schwartz, J, Landis, J, Harlan, W. 1985. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. American Journal of Epidemiology 121: 246–258.

Pocock SJ, Shaper AG, Ashby D, Delves T, Whitehead TP. 1984. Blood lead concentration, blood pressure, and renal function. BMJ (Clin Res Ed). 289: 872–874.

Pocock, S, Shaper, A, Ashby, D, Delves, H, Clayton, B. 1988. The relationship between lead, blood pressure, stroke and heart attacks in middle-aged British men. Environmental Health Perspectives 78: 23-30.

Polverini PJ. 1997. Role of the macrophage in angiogenesisdependent diseases. EXS 79: 11.

Scinicariello F, Yesupriya, A., Chang, M.H., Fowler, B.A. 2010. Modification by ALAD of the association between blood lead and blood pressure in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. Environ. Health PerspectIVE 118 (2), 259–264.

Selbst SM, Sokas RK, Henretig FM, Weller SC, Tershakovec AM. 1993. The effect of blood lead on blood pressure in children. Journal of Environ Pathol Toxicol Oncology. 12; (4) 213-8.

Sharp DS, Becker CE, Smith AH. 1987. Chronic low-level lead exposure: its role in the pathogenesis of hypertension. Med Toxicol. 2:210–232.

Schwartz J. Lead, blood pressure, and cardiovascular disease in men. 1995. Arch Environ Health 50:31-37.

Staessen, J. A, Bulpitt, C. J, Fagard, R, Lauwerys, R. R, Roels, H, Thijs, L, Amery, A. 1994. Hypertension caused by low-level lead exposure: myth or fact? J. Cardiovasc. Risk 1: 87-97.

Staessen, J. A, Roels, H, Fagard, R. 1996a. Lead exposure and conventional and ambulatory blood pressure: a prospective population study. JAMA J. Am. Med. Assoc. 275: 1563-1570.

U.S. EPA. Environmental Protection Agency. 2006. Air Quality Criteria for Lead (Final).U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC, USA.

U.S. EPA. Integrated Science Assessment for lead. EPA/600/R-10/075B, 2012.

Tuomilehto, J. Elo, J, Nissmen, A. 1982. Smoking among patients with malignant hypertension. BMJ. 1:1086.
Vander, A. J. 1988. Chronic effects of lead on the renin-angiotensin system. Environ. Health Perspect. 78: 77-83.

Vaziri ND, Liang K, Ding Y. 1999. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int. 56: 1492–8.

Vaziri, ND, Khan, M. 2007. Interplay of reactive oxygen species and nitric oxide in the pathogenesis of experimental lead-induced hypertension. Clin Exp Pharmacol Physio l34: 920–925.

Vupputuri, S, He, J, Muntner, P, Bazzano, L.A, Whelton, P.K, Batuman, V. 2003. Blood lead level is associated with elevated blood pressure in blacks. Hypertension. 41, 463-468.

Zhang, A; Hu, H; Sánchez, BN; Ettinger, AS; Park, SK; Cantonwine, D; Schnaas, L; Wright, RO; Lamadrid-Figueroa, H; Tellez-Rojo, MM. 2012. Association between Prenatal Lead Exposure and Blood Pressure in Female Offspring. Environ Health Perspectives 120 (3) 445-450.

Table 4-7: Sample Characteristics [(%) or mean ± SD]

Variable	Prishtina (n=21) n (%)	Mitrovica (n=80) n (%)	P-Value
Mean Age	24.96 ± 0.49	24.88 ± 0.48	0.49
Ethnicity			
% Albanian	95.24	68.75	0.01
% Serbian + other	4.76	31.25	
Gender			
% Male	47.62	46.25	0.91
% Female	52.38	53.75	
Education			
% High school or less	19.05	51.25	0.01
% College or more	80.95	48.75	
Employment Status			
% Employed	76.19	47.44	0.02
% Unemployed/searching	23.81	52.56	
Smoking Status			
% Current	66.67	29.33	0.01
% No	33.33	70.67	
Maternal Education			
% High school or less	85.71	93.75	0.22
% College or more	14.29	6.25	
Mean BMI*	23.02 ± 2.34	24.08 ± 4.48	0.15
Mean Weight (kg)	68.58 ± 10.56	70.89 ± 16.64	0.44
Mean Birth weight (g)	3342.5 ± 516.5	3363.3 ± 387.5	0.17
Mean Height (m)	1.72 ± 0.09	1.71 ± 0.09	0.55
Mean Concurrent BPb** (µg/dl)	1.67 ± 0.67	4.91 ± 3.26	<0.0001
Mean Concurrent Hgb*** (g/dl)	14.05 ± 1.68	13.66 ± 1.71	0.36
Mean Concurrent sVCAM-1# (ng/ml)	659.13 ± 170.6	734.39 ± 241.2	0.20
Mean Concurrent sICAM-1^ (ng/ml)	202.40 ± 79.6	220.90 ± 53.75	0.35

*Body Mass Index
**Blood Lead
***Hemoglobin
#Soluble Vascular Adhesion Molecules
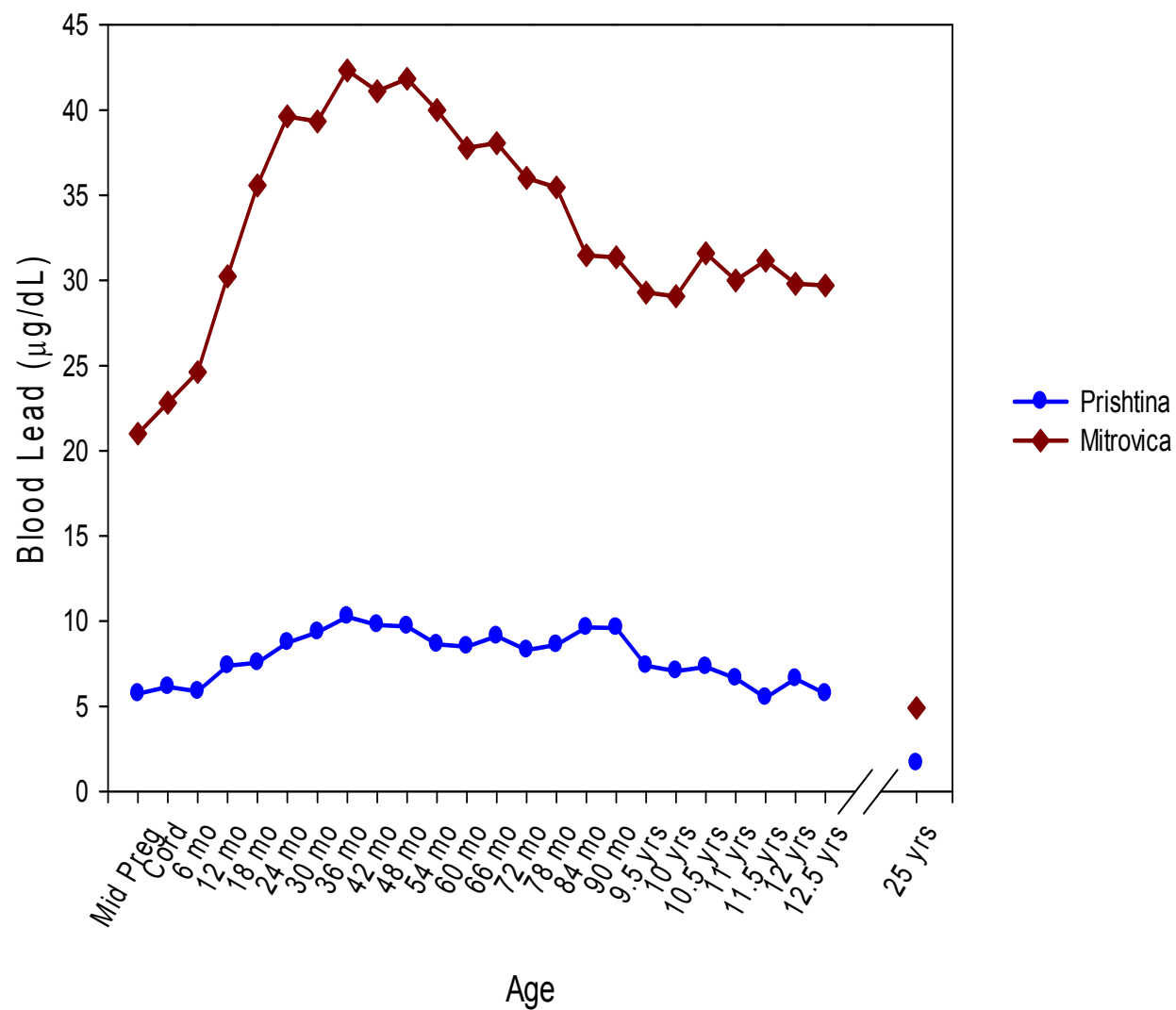^ Soluble Intercellular Adhesion Molecules

Figure 4-8: Average BPb levels in Mitrovica (top) and Prishtina (bottom) for the first 12.5 years of their lives and at 25 years of age (N = 101)

Table 4-8: Demographic and Other Selected Characteristics by Blood Pressure and Blood Lead Levels

	Systolic Blood Pressure			Diastolic Blood Pressure			Blood Lead Levels		
	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
Ethnicity									
Albanian (N=75)	128.51	(9.82)	0.56	80.50	(5.75)	0.43	3.69	(2.8)	0.0028
Serbian and 5 'other' (N=26)	130.36	(22.15)		81.78	(10.12)		5.80	(3.6)	
Gender									
Male (N=47)	131.98	(14.79)	0.04	82.35	(7.49)	0.04	4.56	(3.6)	0.33
Female (N=54)	126.37	(12.74)		79.51	(6.52)		3.95	(2.7)	
Education									
High school or less (N=45)	131.68	(17.62)	0.08	81.36	(8.43)	0.56	5.42	(3.6)	0.0006
College (N=56)	126.82	(9.73)		80.41	(5.86)		3.28	(2.4)	
Smoking									
Current (N=36)	125.83	(11.19)	0.071	79.43	(6.31)	0.13	3.64	(3.1)	0.23
No (N=60)	131.20	(15.38)		81.54	(7.14)		4.44	(3.2)	
BMI									
20-24.9 (N=65)	123.90	(9.67)	0.0001	78.38	(5.36)	0.0001	3.64	(2.7)	0.009
25-29.9 (N=36)	138.59	(15.97)		85.31	(7.86)		5.55	(3.8)	
Location									
Mitrovica (N=81)	129.86	(14.83)	0.20	81.31	(7.49)	0.18	4.91	(3.2)	0.0001
Prishtina (N=21)	125.40	(9.45)		79.00	(5.08)		1.67	(0.6)	

Table 4-9: Linear Regression Models for Current BPb on Systolic and Diastolic Blood Pressure

	Systolic Blood Pressure		Diastolic Blood Pressure	
	β	95% CL	β	95% CL
Unadjusted	1.47	0.64, 2.29	0.57	0.14, 1.00
Adjusted*	1.04	0.25, 1.82	0.33	-0.09, 0.74
Adjusted with smoking	1.41	0.57, 2.25	0.52	0.08, 0.97
Adjusted with BMI	1.05	0.29, 1.81	0.37	-0.03, 0.77
Adjusted with gender	1.39	0.58, 2.21	0.54	0.11, 0.96
Adjusted with ethnicity	1.52	0.66, 2.38	0.57	0.12, 1.02
Adjusted with education	1.37	0.49, 2.24	0.59	0.13, 1.05
Adjusted with all variables**	0.98	0.09, 1.86	0.35	-0.11, 0.81

* adjusted for smoking and BMI.

**adjusted for smoking, BMI, gender, ethnicity, and education
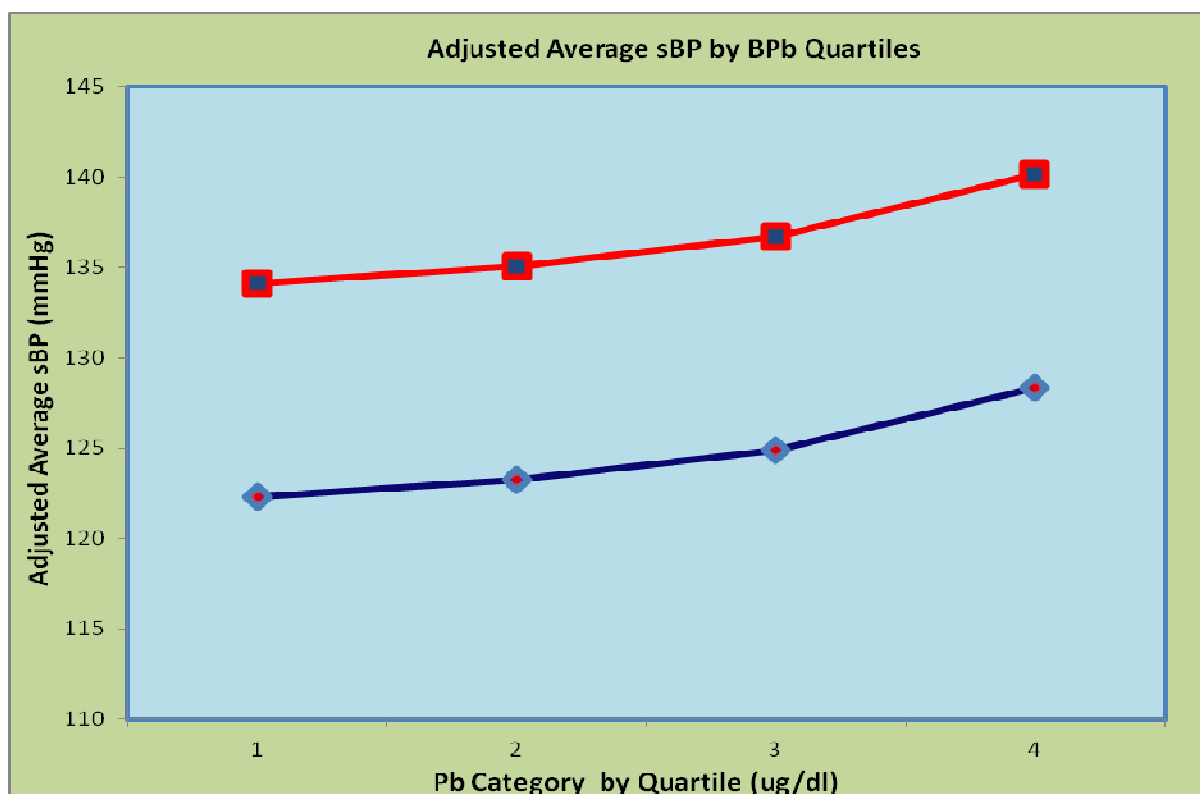
Figure 4-9: Linear relationship of concurrent BPb Quartiles and sBP for: a smoker with high BMI (Upper line-Red) and a non-smoker with low BMI (Lower line-Blue)
 *Adjusted for smoking, BMI, gender, ethnicity, and education

Table 4-10: Linear Regression Models for Current BPb and sVCAM-1 and s-ICAM-1

	sVCAM-1					sICAM-1			
	β	95 % CL	N	R^2			β	95% CL	N	R^2
Unadjusted	12.92	-1.32, 27.17	99	0.022		Unadjusted	4.76	1.14, 8.38	99	0.056
Adjusted *	10.37	-4.44, 25.19	99	0.026		Adjusted *	3.37	-0.42, 7.19	98	0.098
Adjusted #	11.15	-4.03, 26.34	94	0.038		Adjusted #	3.22	-0.61, 7.05	93	0.120
Adjusted ^	11.56	-4.21, 27.33	94	0.027						

* adjusted for ethnicity   ** adjusted for ethnicity and BMI*
 # adjusted for ethnicity and smoking   *# adjusted for ethnicity, BMI, and smoking*
^ adjusted with ethnicity, smoking, and BMI

110
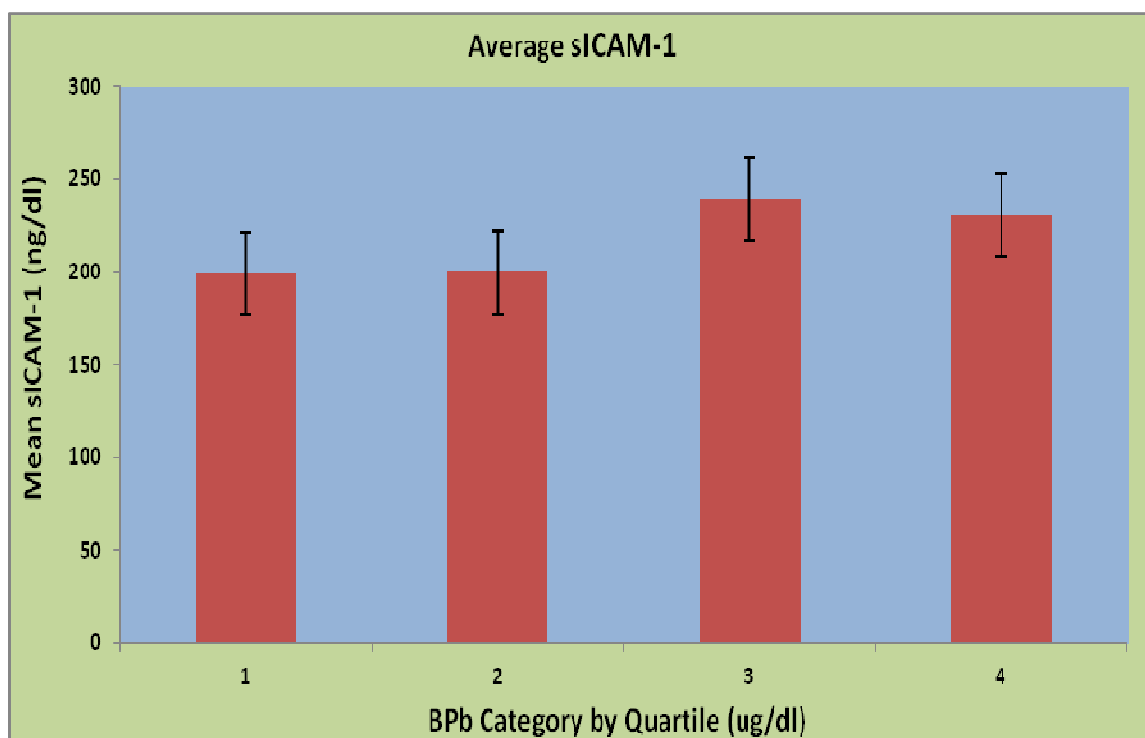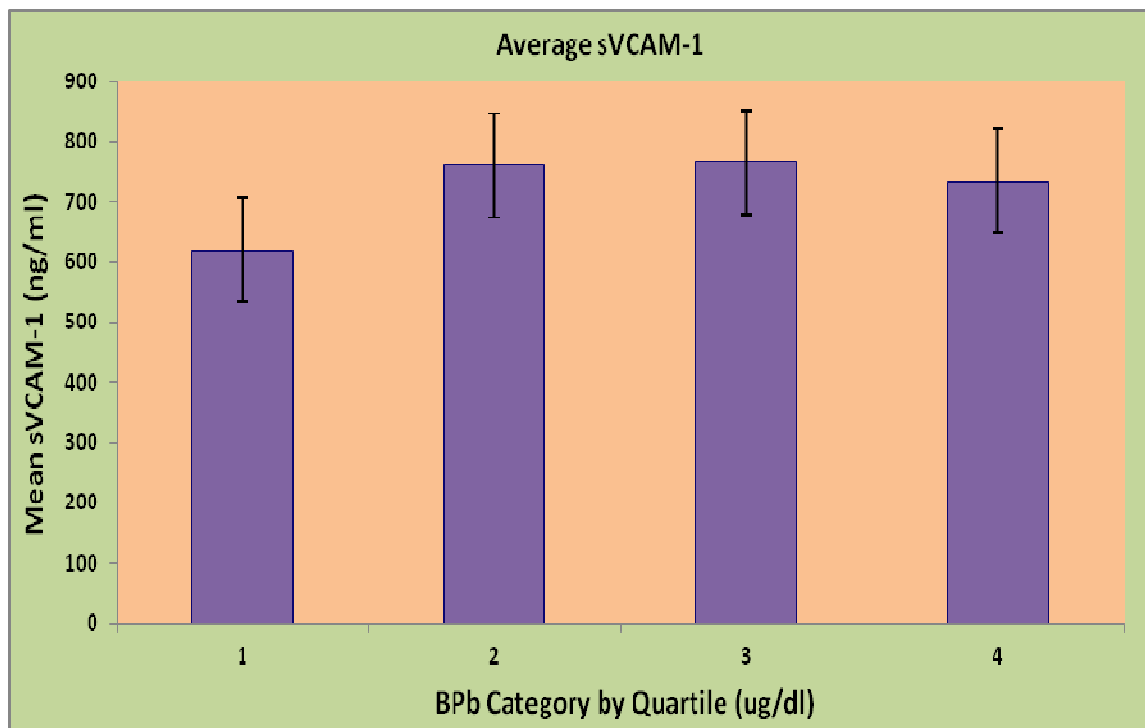
Figure 4-10: Relationship between mean BPb increments by BPb quartiles and sVCAM-1 (top) and sICAM-1 (bottom).
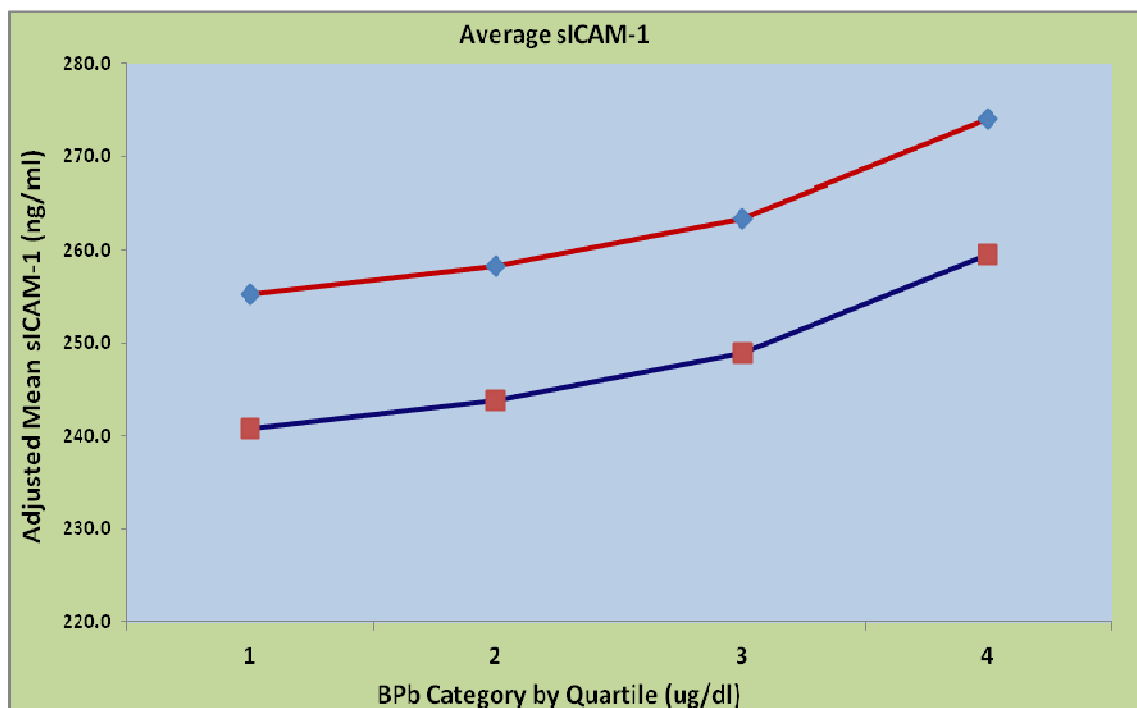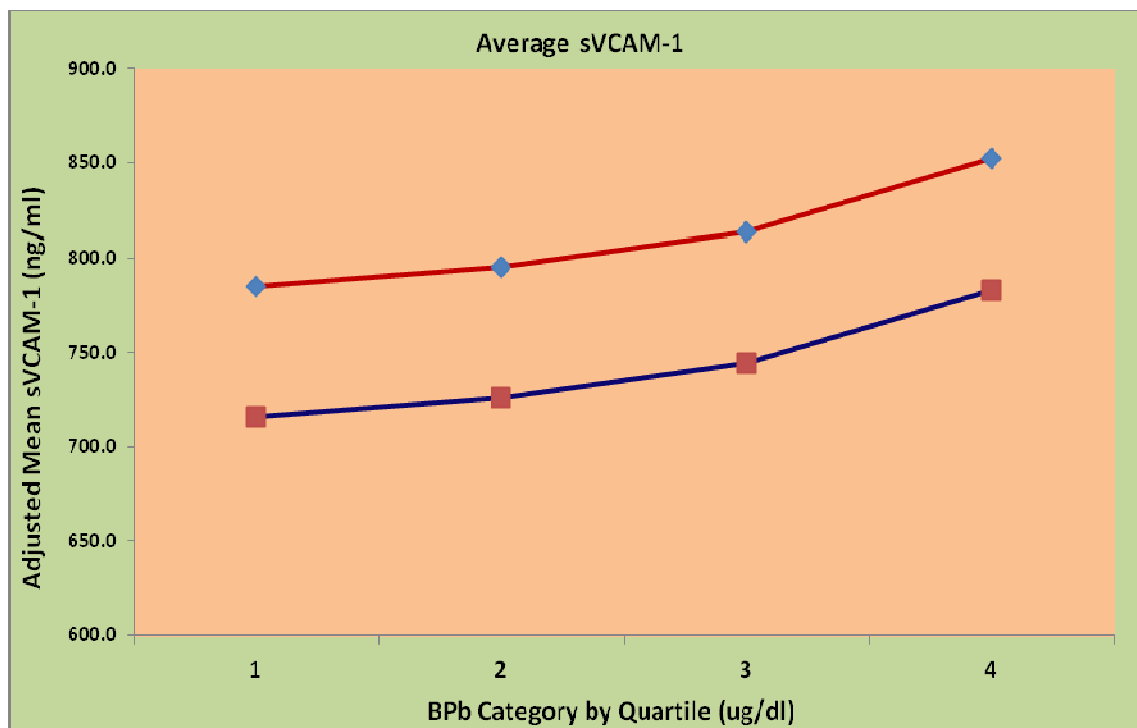
Figure 4-11: Linear relationship between BPb Quartiles and sVCAM-1 (top) and sICAM-1 (bottom).
**The red (upper) lines depict a Serbian, smoker, and high BMI.
***The blue (lower) lines depict an Albanian, smoker, and high BMI.
****Adjusted for smoking, BMI, gender, ethnicity, and education.

CHAPTER V: Long-Term Effects of Environmental Lead on Erythropoietin Production in Young Adults: A Follow-up Study of a Prospective Cohort in Kosovo

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Abstract

ABSTRACT: Epidemiologic studies examining the relationship between environmental lead

(Pb) exposure and erythropoietin (EPO) production have reported contrasting findings. Most

epidemiological studies evaluating this relationship are predominantly cross-sectional and report

different findings. It is unknown however, if exposure to Pb earlier in life has an effect on EPO

production later in life. Here we evaluate this relationship prospectively.

OBJECTIVE: We investigated the association between prenatal, early childhood, and

concurrent Pb exposure and EPO production later in life.

METHODS: From our original prospective birth cohort study that was performed in Mitrovica

(a mining town) and Pristina, Kosovo, from 1985-1998, we located and assessed BPb and serum

EPO in 101 participants (mean age 24.9 years old).   We examined the association between

concurrent blood lead (BPb) and EPO. Pb exposure was operationalized as concurrent BPb

levels, and then as a series of cumulative lifetime exposure periods measured from the prenatal

period onward through 12.5 years of age.

RESULTS: BPb levels, measured every six months from birth through age 12, varied widely

across the study sample and had a wide range of levels early in life. At age 25, the mean BPb

was 4.91 µg/dl, with a range of 1.41-16.4 µg/dl in Mitrovica and mean BPb of 1.68 µg/dl with a

range of and 0.69-3.51 µg/dl in Prishtina. The crude model [unadjusted for hemoglobin (Hgb)

status] shows no relationship between concurrent BPb and EPO among all study participants.

However, once adjusted for Hgb levels, a statistically significant association (β=0.19) between

log BPb and log EPO is observed (95% CI 0.06, 0.31). Also, a subsequent introduction of an

interaction term between BPb and Hgb strengthens the relationship between log BPb and EPO (β = 0.41; 95% CI 0.13, 0.70). The results presented here are different from the findings in this cohort when the study participants were 12.5 years of age.

CONCLUSION: Current study results, along with previously reported findings on this cohort, suggest that a dramatic reduction of Pb exposure may allow for a reversal of the impact that prolonged Pb exposure may have on EPO production.

KEY WORDS: Blood lead, erythropoietin, Kosovo, environmental lead exposure.

Background

      Environmental lead (Pb) exposure has long been known to be associated with anemia

(Aub et al., 1925; Baker et al., 1979; Schwartz et al., 1990), likely due to a range of mechanisms

including shortened erythrocyte survival (Leikin et al. 1963; Hernberg et al., 1967), ineffective

erythropoiesis (Berk et al., 1970), and inhibition of enzymes of the heme synthetic pathway

(Lichtman et al., 1963; Piomelli et al., 1975; Piomelli et al., 1982). Even in Pb-exposed

individuals with normal hemoglobin (Hgb) levels, there is evidence of sub-clinical effects on

hematopoiesis. For example, Grandjean et al (1989) reported that following the donation of a

unit of blood, Pb-workers demonstrated "delayed blood regeneration" in comparison to non-

worker controls. That report led us to hypothesize that the production of erythropoietin (EPO),

the renal hormone that regulates red cell production, may be inhibited by Pb exposure; EPO is

primarily produced in the proximal renal tubules (Caro et al., 1984; Erslev et al., 1986) where Pb

is known to accumulate. We subsequently demonstrated that, as compared to controls, serum

EPO levels declined as BPb increased in a population of chronically exposed pregnant women

with a wide range of blood Pb (BPb) and Hgb levels (Graziano et al., 1991). The latter study

took place in two towns in Kosovo (then Yugoslavia): Mitrovica, the site of Pb mining and

smelting operations; and Prishtina, a relatively unexposed city 25 miles away.

      We subsequently described pregnancy outcome among 1,502 women in those two towns

(Murphy et al., 1990; Factor-Litvak et al., 1991) and went on to study childhood development

through age 12 years in a subset of their offspring (Wasserman et al., 1992, 1994, 1997). In

addition, we went on to examine the relationship between BPb and serum EPO in the children at

ages 4.5, 6.5 and 9.5 years. We reported that at age 4.5, children required a *hyperproduction* of

EPO to maintain normal Hgb levels, but with increasing age (and exposure from the smelter

operations) this compensatory mechanism gradually failed, perhaps indicative of cumulative effects of Pb on renal endocrine function (Factor-Litvak et al, 1998).

In the current report we had the opportunity to re-examine a subset of this pediatric cohort, now 25 years of age, to test the hypothesis that – as in their mothers - chronic environmental Pb exposure would lead to an inability to mount an increase in EPO when Hgb is relatively low. During the interim, however, Pb exposure (and BPb levels) declined dramatically as a result of the shutdown of the Mitrovica Pb industries.

Methods

Ethical Approvals: This study was approved by the Columbia University Medical Center IRB and by the "Komiteti Etik", an NIH-registered IRB at the University of Prishtina, Kosovo.

Study Design: By various means we located and identified 101 members of the original cohort in Kosovo and requested their participation in a follow-up study, in which each participant would be evaluated once to assess their current BPb, serum EPO, and Hgb levels. Appointments were arranged for participants to report to a central location in each town of the two towns. Questionnaires were administered in the participant's primary language, Albanian or Serbian. A trained laboratory technologist and a physician carried out the field work. All blood samples were refrigerated immediately at 4º Celsius then frozen at $-20^{o}C$ prior to shipment to Columbia University for analysis.

Demographic information and other cohort characteristics were collected via questionnaire. In addition, we utilized the information from their mothers' mid-pregnancy and delivery questionnaires, and their own childhood questionnaires to ascertain the life-style characteristics of the cohort members as needed. While we contacted and recruited study participants in both towns, we encountered more challenges finding and recruiting the participants from Prishtina. This may be due to the fact that the city expanded greatly in the past 8-10 years with a number of changes especially in the ethnic makeup of the population. In addition, once contacted, those in Prishtina were less interested, and more of them refused to take part in the study. This may be due to the fact that they did not view the study as beneficial to them as did those in Mitrovica. Another factor that may have influenced the recruitment may have been that the overwhelming majority of the pre-war Serbian population in Prishtina had been uprooted from Kosovo at the beginning of NATO campaign in 1999. However, the Serbian population in Mitrovica (Northern part of the city) for the most part has remained in place. There may have been some temporary migration during the few months at the height of the war, but it has been reported that most had returned to their homes shortly after. Similarly, the Albanian population in Mitrovica has remained very much intact with similar pattern of temporary migration during the few months (1-3 months) of the height of the hostilities. Their temporary migration was mostly to camps in neighboring Albania and Montenegro. However, as the hostilities diminished, the overwhelming majority returned to their homes.

Blood samples were collected by venipuncture in EDTA vacutainers. Hgb concentrations were measured using by the standard cyanmethemoglobin method. An enzyme linked immunosorbent assay kit (ELISA) was used for the quantitative determination of serum-

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EPO concentrations (Quantikine IVD Human Erythropoietin), according to the manufacturer's instructions.   BPb levels were analyzed using a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), model AAnalyst 600 (Perkin-Elmer, Shelton, CT) using a method modified after Fernandez and Hilligoss (1982). Quality Control (QC) samples were run daily, at the beginning of the run, after every 10th sample, and at the end of the run.   Columbia University's laboratory participates in a Center for Disease Control quality control program for BPb; in the past three years, the agreement statistic (interclass correlation) between the expected and observed BPb was 0.99. BPb measurements from childhood were already available from the parent cohort study, measured every six months from birth through age 12 years; those measurements were conducted in the same laboratory using the same method.

Statistical Analyses

We first conducted descriptive analyses to compare the distributions of variables of interest by utilizing t-test or chi-square tests, and simple bivariate linear and logistic models. Prior to fitting multivariable models, simple linear models estimated the crude associations between current BPb levels with EPO. We used linear regression models to examine the association between concurrent BPb and EPO controlling for concurrent Hgb concentration, the most important predictor of EPO (Factor-Litvak et al., 1998). BPb measures were available for each subject from birth through 12.5 years of age, and at the follow up. We first examined the associations with BPb measured at the time of follow up. Second, we calculated cumulative lead exposure, using the trapezoidal area under the curve for various age periods: from birth through age 2, ages 2- 4, 4-7, and ages 7-12. Third, we also examined the associations between EPO and the total area under the BPb vs. age curve. The distribution of BPb was not normally distributed

so we utilized a base 10 logarithmic transformation. In addition, we controlled for the potential

confounders including, ethnicity, gender, body mass index (BMI), smoking, employment status,

and education. Potential confounders were retained in the final models if the estimated

coefficient relating BPb to EPO changed at least 10% with their inclusion. All analyses were

performed using SAS version 9.3 (SAS Institute, Carey, NC).

Results

We first compared the characteristics of the current study sample of 101 participants to

those of the original birth cohort of 576 participants. In comparison to those seen at birth, the

current sample was more likely to be Albanian and reside in Mitrovica (data not shown). The

characteristics of the current Prishtina and Mitrovica participants are presented in **Table 5-11**.

On average, those from Prishtina were more likely to be Albanian, have a college education, be

employed and smoke cigarettes. Mean Hgb was 0.39 g/dl lower in Mitrovica than Prishtina, but

this difference was not significant. However, the mean serum EPO concentration of 10.99

mIU/ml in Mitrovica was significantly higher than that of 8.49 mIU/ml in Prishtina ($P < 0.04$).

Overall, 17% of participants had Hgb <11.0 g/dL and ~32 % had Hgb < 13 g/dL.

In **Table 5-12** we present the mean values of BPb, Hgb and EPO of the current sample as

compared to those of all children in the cohort at birth and at ages 2, 7 and 12 years. With one

exception, the mean values of BPb, Hgb and EPO of the current sub-sample did not differ from

the original study sample; the exception is that at age 2, the current study sample had lower Hgb

levels than the overall sample (10.64 vs 11.13 g/dl, respectively; $p = 0.017$)

Not surprisingly, the mean concurrent BPb of 4.91 µg/dl in Mitrovica was significantly

higher than that of 1.67 µg/dl in Prishtina ($P < 0.0001$). Figure 5-12 illustrates the lifetime BPb

histories of these 101 participants. Peak BPbs occurred at 36 months of age in both towns, with peak values of 42.3µg/dl in Mitrovica and 10.3 µg/dl in Prishtina.   Obviously, a dramatic fall in BPb has occurred since the participants were last seen at age 12.5 years.

Hgb concentration is the strongest predictor of serum EPO levels. We therefore first examined the relationship between BPb and serum EPO by stratifying the sample into quartiles of Hgb **(Figure 5-13)** and also stratifying by those above and those below the median BPb concentration of 3.27 µg/dl. As shown in the figure, within each Hgb quartile, those with higher BPb values tended to have higher serum EPO concentrations, most notably in the lowest quartile of Hgb, where the physiologic demand for compensatory EPO synthesis is most pronounced. A simple linear regression model relating log BPb to log serum EPO did not find a significant relationship between the two variables ($\beta = 0.05$; 95% CI -0.09, 0.20) **(Table 5-13)**. However, when adjusted for Hgb as a continuous variable, the relationship became significant ($\beta = 0.19$; 95% CI 0.06, 0.31). This finding is illustrated graphically in **Figure 5-14**. The association between BPb and EPO varied by anemia status. For those with Hgb levels < 12.5, the estimated increase in EPO is 0.41 mIU/ml (95% CI 0.13, 0.70) per log-unit of BPb. However, for those with Hgb levels $\geq$ 12.5, the estimate is 0.05 mIU/ml (95% CI -0.10, 0.20) per log-unit of BPb. These findings are illustrated graphically in **Figure 5-15**, which illustrates the relationship between log BPb and log EPO dichotomized by Hgb (less than or greater than or equal to 12.5 g/dl). In the multivariable analysis, significant positive associations were found between concurrent EPO and estimates of cumulative Pb exposure (i.e., AUC) between birth and age 2, ages 2- 4, ages 4-7. The estimated regression coefficients declined in magnitude as the children aged. At age 7-12 no significant associations between cumulative lead exposure and EPO were found, however, the sign of the estimated beta changed to negative (data not shown).

Discussion

The participants in the current study visit had last been evaluated in 1998 at age 12.5 years. By 2011, at age 25, their mean BPbs had fallen from 29.7 to 4.91 µg/dL in Mitrovica, and from 5.73 to 1.67 µg/dL in Prishtina. By that time the operations at "Trepca Mines and Smelters" in Mitrovica had been completely shut down for more than a decade, undoubtedly leading to a reduction of airborne Pb and ongoing exposure. Nevertheless, the legacy of early childhood Pb exposure was still apparent at the age of 25, as evidenced by the mean BPb of 4.91 µg/dL, and range of 1.41-16.4 µg/dL, in that town.

In this study we examined the relationship between environmental Pb exposure and serum EPO in a group of young adults in whom this relationship has been followed longitudinally over time. We tested the hypothesis that higher levels of prenatal and early life Pb exposure are associated with decreased EPO production later in life. Lead exposure was estimated using past measurements of BPb from birth through age of 12, which were already available, as well as concurrent BPb at 25 years of age. The results show a positive association between concurrent BPb and serum-EPO levels with a significantly more pronounced slope in the regression line for the low-Hgb level participants. These results resemble the findings in the original full cohort at 4.5 and 6.5 years of age, at which time we reported that the maintenance of a normal Hgb required hyperproduction of EPO to do so, presumably a physiologic adaptation to shortened red cell survival in the face of high BPbs in the Mitrovica participants. In contrast, when the original cohort was 9.5 and 12 years of age, they were no longer capable of doing so, suggestive of cumulative toxicity to the peritubular cells of the kidney that are responsible for EPO synthesis (Graziano et al., 2004). In addition, the current EPO findings also contrast those reported in the anemic mothers of this study cohort (Graziano et al., 1991) during pregnancy,

where serum EPO levels were lower in those with higher BPb levels; those women, however, had high concurrent BPb levels, ranging from 23.1 to 36.2 ug/dL

The current findings indicate that there is a positive association between concurrent BPb and serum EPO, a finding that is particularly apparent in those with relatively lower Hgb concentrations **(Figures 5-13 and 5-14)**. We interpret these findings to suggest that the dramatic decline in BPb concentrations that occurred during the many years since this cohort was last evaluated has allowed renal peritubulal cell function to recover. Despite the relatively low concurrent BPbs at age 25, the observed positive association between BPb and serum EPO suggest that perhaps Pb-induced effects on circulating red cells lead to shortened red cell survival, thereby creating a demand for endogenous EPO synthesis as a compensatory mechanism. Alternatively, it is conceivable that Pb has a lasting adverse effect on erythroid progenitor cells that leads to an inappropriate demand for EPO (Osterode et al, 1999).

Increases in BPb have been reported to be associated with decreased serum EPO levels in Pb workers with BPbs ranging from 30-92 ug/dL (Romeo et al, 1996), as compared to controls with a mean BPb of 10 ug/dL. A similar negative association has been reported in children by Liebelt and co-workers (1999), who performed regression analyses examining the relationship between BPb and EPO (controlling for Hgb) in 86 children with a range of BPbs of 2-84 ug/dL. However, the *positive* association between BPb and EPO in the current study suggests that the relatively low concurrent BPbs are not sufficient to impair EPO synthesis.

Some of the strengths of this study include the wide range of BPb levels measured at multiple time-points early in life and the range of Hgb levels. Limitations include the fact that the results might not be generalizable to other populations with different profiles or risk factors,

and that we were only able to reassess a limited number of participants from the original cohort. In addition, another limitation is the 12 ½ year gap in the BPb measurements.

In summary, we report that in this group of young adults who were chronically exposed to Pb in early childhood, serum EPO concentrations responded appropriately as a function of Hgb concentration, but also increased inappropriately as concurrent BPb increased. Thus, a perturbation of this renal/hematopoietic balance is still evident many years after the cessation of exogenous Pb exposure.

REFERENCES

Aub JC, Fairhall LT, Minot AS, Reznikoff P. 1925. Lead poisoning. Medicine 4:1-250.

Baker EL Jr, Landrigan PJ, Barbour AG, et al.(1979) Occupational lead poisoning in the United States: clinical and biochemical findings related to blood lead levels. Br J Ind Med. 36:314–322.

Baker EL Jr, Landrigan PJ, Barbour AG, et al. 1979. Occupational lead poisoning in the United States: clinical and biochemical findings related to blood lead levels. Br J Ind Med. 36:314–322

Berk PD, Tschudy DP, Shepley LA, Waggoner JG, Berlin NI. 1970. Hematologic and biochemical studies in a case of lead poisoning. Am J Med. 48:137–144.

Caro J, Erslev AJ. 1984. Biologic and immunologic erythropoietin in extracts from hypoxic whole rat kidneys and their glomerular and tubular fractons. Journal of Laboratory and Clinical Medicine; 103:922-931.

Erslev AJ, Caro J. 1986. Physiologic and molecular biology of erythropoietin. Med Oncology Tumor Pharmacother 3:159-164

Factor-Litvak P, Graziano JH, Kline J, Popovac D,Mehmeti A, Ahmedi G, Shrout P, Murphy MJ, Gashi E,Haxhiu R. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. Int J Epidemiology, 20:722-728 (1991).

Factor-Litvak, P, Slavkovich, V, Liu, L, Popovac, D, Preteni, E, Capuni-Paracka, S, Hadzialjevic, S, Lekic, L, LoIacono, N, Kline, J, and Graziano, JH. 1998. Hyperproduction of erythropoietin in non-anemic lead-exposed children. Environmental Health Perspectives. 106; 6, 361-364.

Fernandez, F, Hilligoss. 1982. An improved graphite furnace method for the determination of lead in blood using matrix modification and L'vov platform. Atomic Spectroscophotometry 3, 130-131.
Grandjean P, Jensen BM, Sando SH, Jogensen PJ, Antonsen S. 1989. Delayed blood regeneration in lead exposure: an effect on reserve capacity. Am J Public Health 79:1385-1388).

Graziano, JH, Slavkovic, V, Factor-Litvak, P, et al. 1991. Depressed serum erythropoietin in pregnant women with elevated blood lead. Archives of Environmental Health 46: 347-350.

Graziano, JH, Slavkovich, V, Liu, X, Factor-Litvak, P, Todd, A. 2004. A prospective study of prenatal and childhood lead exposure and erythropoietin production. Journal of Occupational and Environmental Medicine 46: 924-929.

Hernberg S, Nurminen M, Hasan J. 1967. Nonrandom shortening of red cell survival times in men exposed to lead. Environ Res. 1:247–261.

Leikin S, Eng G. 1963. Erythrokinetic studies of the anemia of lead poisoning. Pediatrics. 31:996–1002.

Lichtman HC, Feldman F. 1963. In vitro pyrroland porphyrin synthesis in lead poisoning and iron deficiency. J Clin Invest. 42:830–839.

Liebelt, E.L., Schonfeld, D.J., Gallagher, P. 1999. Elevated blood lead levels in children are associated with lower erythropoietin concentrations. Journal of Pediatricds 134, 107-109.

Murphy, MJ, Graziano, JH, Popovac, D, Kline, J, Mehmedi, A, Factor-Litvak, P, Ahmedi, G, Shrout, P, Rajovic, B, Nenezic, DU, Stein, ZA. 1990. Past Pregnancy Outcomes among Women Living in the Vicinity of a Lead Smelter in Kosovo, Yugoslavia. Amer J Pub Health 80: 33-35.

Osterode W, Barnas U, Geissler K. 1999. Dose dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead: new aspects of anaemia induced by lead. Occup Environ Med. 56:106–109.

Piomelli S, Lamola AA, Poh-Fitzpatrick MB, Seaman C, Harber LC. 1975. Erythropoietic protoporphyria and lead intoxication: the molecular basis for difference in cutaneous photosensitivity. I. Different rates of disappearance of Protoporphyrin from the erythrocytes, both in vivo and in vitro. J Clin Invest. 56:1519–1527.

Piomelli S, Seaman C, Zullow D, Curran A, Davidow B. 1982. Threshold for lead damage to heme synthesis in urban children. Proceedings of the national Academy of Sciences, USA, 79 3335-3339.

Romeo, R., Aprea, C., Boccalon, P., Orsi, D., Porcelli, B., Sartorelli, P. 1996. Serum erythropoietin and blood lead concentrations. Int. Arch. Occup. Environ. Health 69, 73-75.

Schwartz J, Landrigan PJ, Baker EL Jr, Orenstein WA, von Lindern IH. 1990. Lead induced anemia: dose-response relationships and evidence for a threshold. Am J Public Health. 80:169–172.

Wasserman G, Graziano JH, Factor-Litvak P, Popovac D, Morina N, Musabegovic A, Vrenezi N, Capuni-Paracka S, Lekic V, Preteni-Redjepi E. 1992. Independent effects of lead exposure and iron deficiency on developmental outcome at age 2 years. J Pediatr 121:695-703.

Wasserman G, Graziano JH, Factor-Litvak P, Popovac D, Morina N, Musabegovic A, Vrenezi N, Capuni-Paracka S, Lekic V, Preteni-Redjepi E. 1994. Consequences of lead exposure and iron supplementation on childhood development at age 4 years. Neurotoxicol Teratol 16:233-240

Wasserman GA, Liu X, Lolacono NJ, Factor-Litvak P, Kline JK, Popovac D, Morina N, Musabegovic A, Vrenezi N, Capuni-Paracka S, et al. 1997. Lead exposure and intelligence in 7-year-old children: the Yugoslavia prospective study. Environ Health Perspect 105:956-962

Table 5-11. Sample Characteristics [(%) or mean ± SD]

Variable	Prishtina (n=21) n (%)	Mitrovica (n=80) n (%)	P-Value
Mean Age	24.96 ± 0.49	24.88 ± 0.48	0.49
Ethnicity			
% Albanian	95.24	68.75	0.01
% Serbian + other	4.76	31.25	
Gender			
% Male	47.62	46.25	0.91
% Female	52.38	53.75	
Education			
% High school or less	19.05	51.25	0.01
% College or more	80.95	48.75	
Employment Status			
% Employed	76.19	47.44	0.02
% Unemployed/searching	23.81	52.56	
Smoking Status			
% Current	66.67	29.33	0.01
% No	33.33	70.67	
Maternal Education			
% High school or less	85.71	93.75	0.22
% College or more	14.29	6.25	
Mean BMI*	23.02 ± 2.34	24.08 ± 4.48	0.15
Mean Weight (kg)	68.58 ± 10.56	70.89 ± 16.64	0.44
Mean Birthweight (g)	3342.5 ± 516.5	3363.3 ± 387.5	0.17
Mean Height (m)	1.72 ± 0.09	1.71 ± 0.09	0.55
Mean Concurrent BPb**(µg/dl)	1.67 ± 0.67	4.91 ± 3.26	<0.0001
Mean Concurrent Hgb***(g/dl)	14.05 ± 1.68	13.66 ± 1.71	0.36
Mean Concurrent EPO^ (mIU/ml)	8.49 ± 3.74	10.99 ± 7.12	0.04

Body Mass Index
Blood Lead
***Hemoglobin*
^Erythropoietin

Table 5-12: Sample biomarker characteristics (N=576) compared to current follow-up cohort of young adults (N=101)

Biomarkers	**Prishtina**		**Mitrovica**	
Mean BPb (µg/dl)	**Original Cohort**	**Current sample (N=21)**	**Original Cohort**	**Current sample (N=80)**
BPb-Umb. Cord	5.66 (3.5)* [255]**	6.14 (3.5) p-0.54	21.95 (7.9) [308]	22.8 (7.9) p-0.39
BPb-Age 2 years	9.31 (4.8) [170]	8.74 (5.3) p-0.61	37.52 (12.3) [190]	39.6 (12.4) p-0.20
BPb-Age 7 years	7.56 (3.7) [100]	9.61 (7.5) p-0.07	31.15 (10.5) [102]	31.4 (10.0) p-0.87
BPb-Age 12 years	6.31 (2.0) [61]	6.61 (3.0) p-0.60	29.89 (9.7) [49]	29.8 (8.7) p-0.58
Mean Hgb (µg/dl)	**Original Cohort**	**Current sample**	**Original Cohort**	**Current sample**
Hgb - Cord	16.32 (2.4) [255]	15.90 (2.3) p-0.44	15.89 (2.2) [308]	15.48 (2.3) p-0.14
Hgb - Age 2 years	10.76 (1.3) [170]	10.64 (1.6) p-0.69	11.13 (1.5) [190]	10.64 (1.6) p-0.01
Hgb - Age 7 years	12.72 (0.8) [100]	12.84 (1.0) p-0.55	12.83 (0.9) [102]	12.84 (1.0) p-0.94
Hgb - Age 12 years	13.05 (0.9) [60]	12.88 (1.0) p-0.47	12.77 (0.8) [49]	12.88 (1.0) p-0.51
Mean EPO (mlU/ml)	**Original Cohort**	**Current sample**	**Original Cohort**	**Current sample**
EPO - Age 4.5 years	5.6 (3.1) [97]	7.15 (6.3) [9] p-0.20	7.8 (7.3) [114]	8.6 (7.6) [23] p-0.63
EPO - Age 6.5 years	8.2 (4.1) [83]	6.99 (2.4) [13] p-0.30	9.7 (4.1) [95]	9.2 (3.8) [42] p-0.50
EPO - Age 9.5 years	8.8 (6.4) [113]	7.68 (3.7) [12] p-0.55	8.8 (6.2) [121]	8.5 (3.5) [53] p-0.74
EPO - Age 12 years	9.2 (3.4) [98]	10.61 (6.0) [14] p-018	9.6 (3.9) [111]	9.81 (6.1) [62] p-0.78

*( ) Standard Deviation
** [ ] No. of the cohort participants
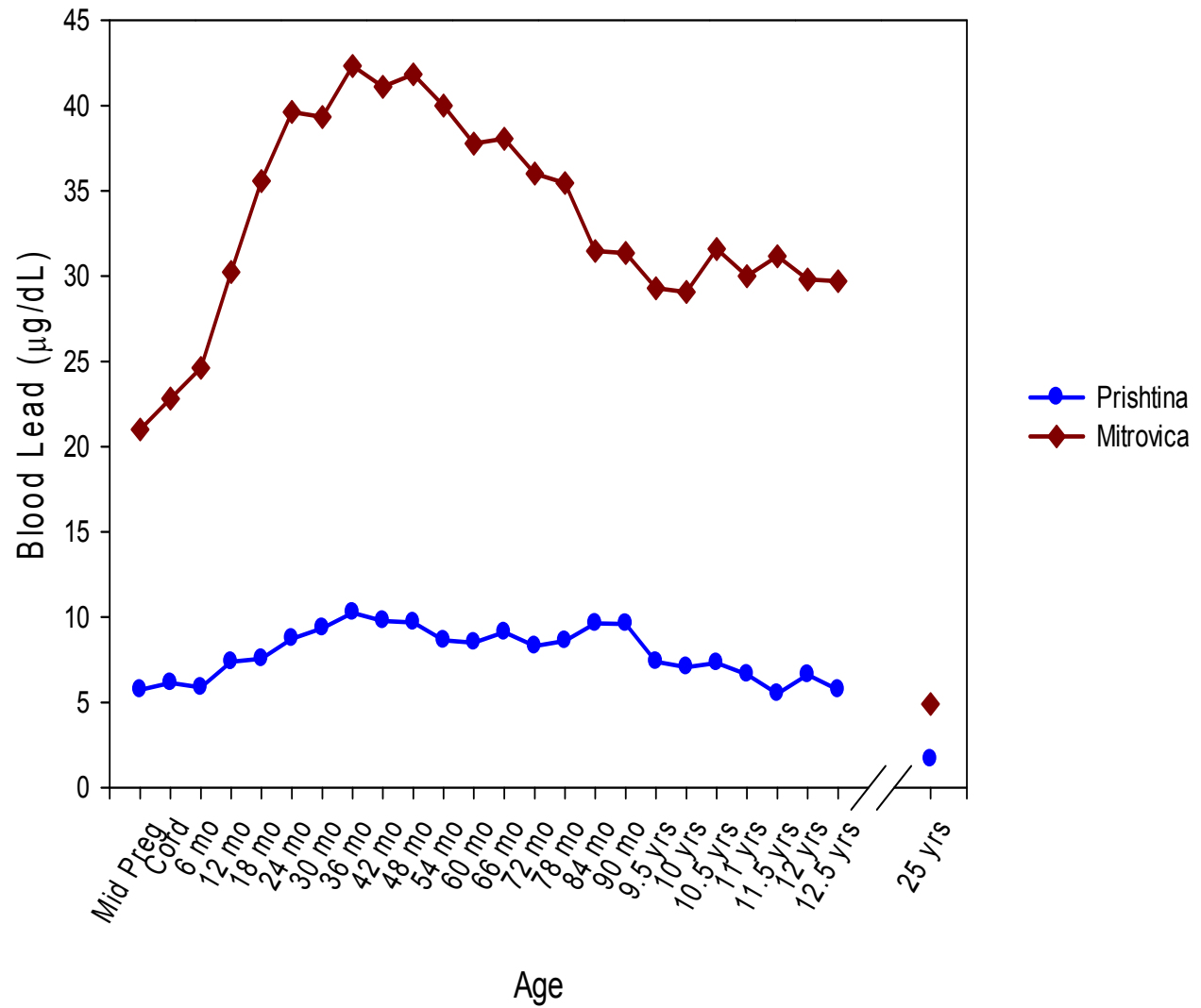
Figure 5-12: Average BPb levels in Mitrovica (top) and Prishtina (bottom) for the first 12.5 years of their lives and at 25 years of age (N = 101)
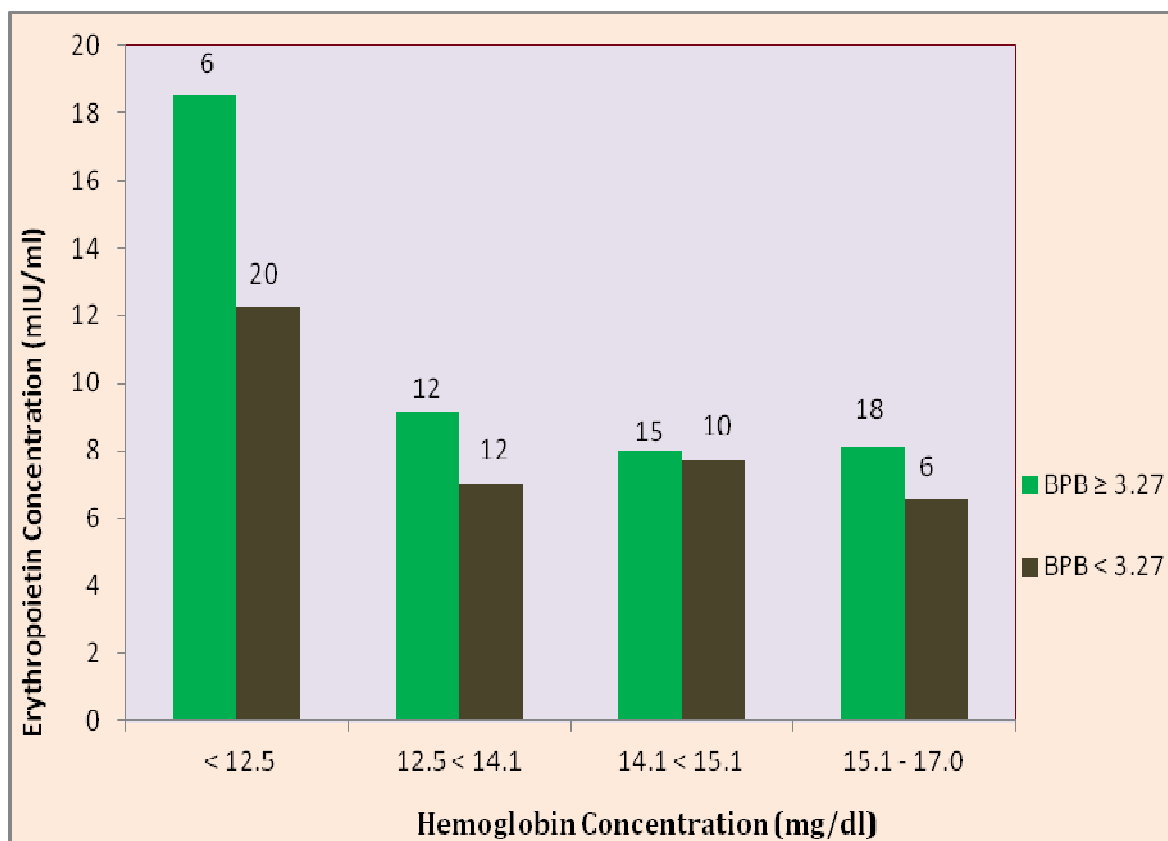
Figure 5-13: Mean EPO concentrations stratified by Hgb concentrations. Numbers above bars indicate the number of participants each bar represents.

Table 5-13: Linear Regression Models Relating (log) BPb to (log) Serum Erythropoietin Concentrations (N=101)

	β	95% CL
Unadjusted	0.05	-0.09, 0.20
Adjusted *	0.19	0.06, 0.31
Adjusted ^		
^Anemic (Hgb < 12.5 g/dl)	0.41	0.13, 0.70
^Non-Anemic (Hgb > 12.5 g/dl)	0.05	-0.10, 0.20

adjusted for hemoglobin (continuous).
^ adjusted for hemoglobin (dichotomous) with interaction term of lead (continuous) and hemoglobin (dichotomous).
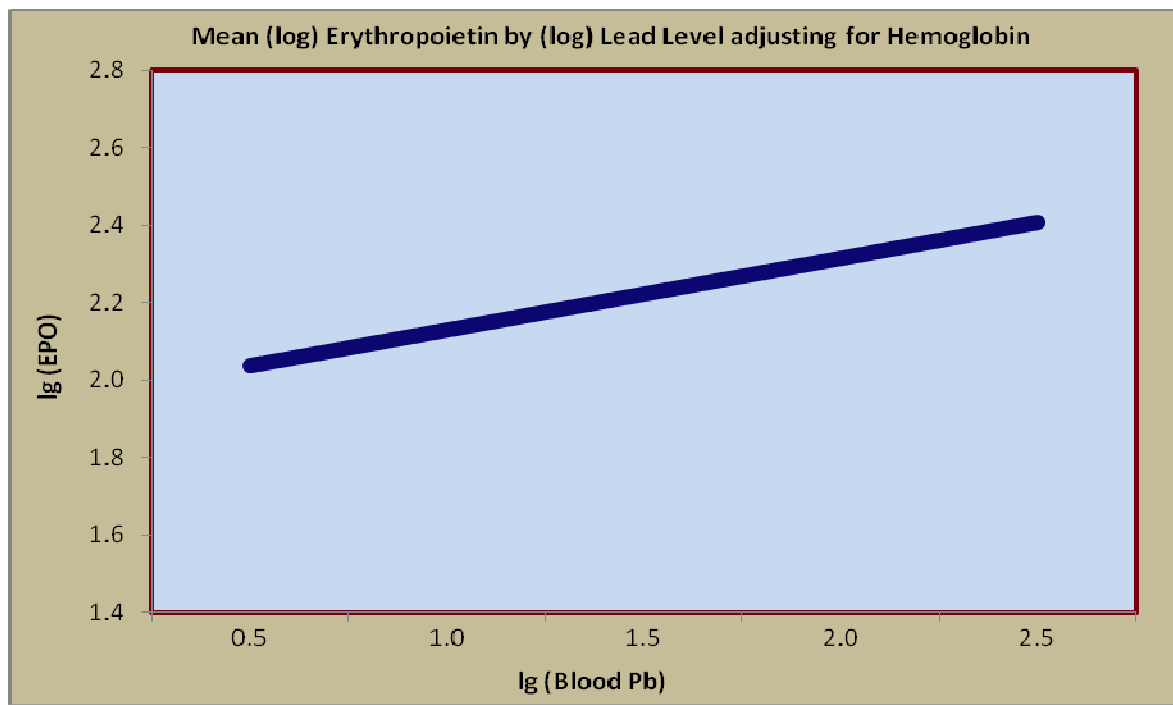
Figure 5-14: Relationship between log(BPb) and log(EPO) adjusted for hemoglobin
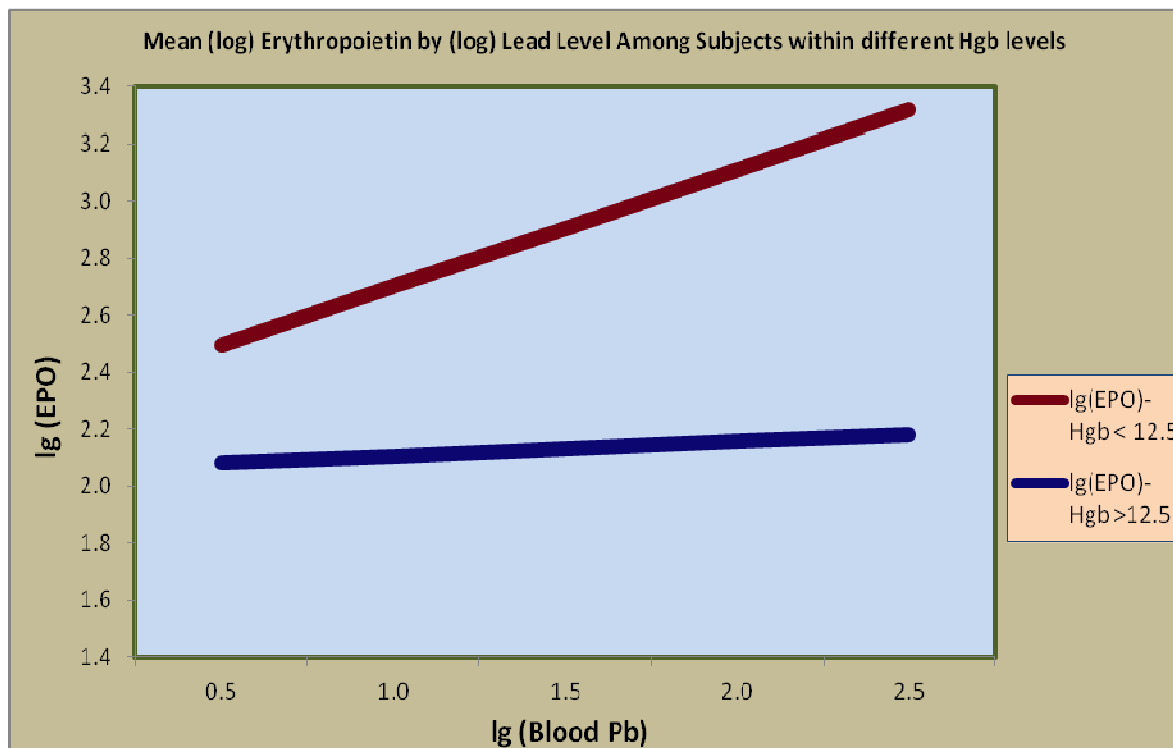
Figure 5-15: Relationship between log(BPb) and log(EPO) in anemic and non-anemic subjects

CHAPTER VI: CONCLUSIONS and FUTURE DIRECTIONS

6.1 Summary of Results

      The studies comprising this dissertation have evaluated the association between prenatal, early childhood, and concurrent Pb exposure and BP, serum levels of circulating sICAM-1 and sVCAM-1, and EPO production later in life. For these studies we recruited a subset of participants (N=101) from a larger birth cohort that was followed from 1985-1998. In that study, a group of 1,502 pregnant women were recruited for a prospective evaluation of the relationship between environmental Pb exposure and birth outcomes (Graziano et al, 1990; Murphy et al., 1990; Factor-Litvak et al, 1991); the study took place in two towns in Kosovo (then part of the former Yugoslavia): Mitrovica (a mining town) and Prishtina (a non-mining town). Subsequently, a representative group of 541 of their newborns were selected for long-term prospective follow up. The children were followed longitudinally at six-month intervals for 12 years to examine the effects of environmental Pb exposure on a variety of health outcomes including cognitive and motor function, anemia, endocrine function and growth.   Follow up rates over time were excellent in that 70% of the total cohort was evaluated at 6 years of age, and 65% were evaluated at 12 years of age, at which point the study was - until now - concluded. From that original prospective birth cohort study, we located and assessed BPb, BP, circulating levels of sICAM-1 and sVCAM-1, and serum EPO in 101 participants (mean age 24.9 years old).

      The outcomes studied for this dissertation that are discussed below included effects of Pb on BP, circulating levels of sICAM-1 and sVCAM-1, and EPO production.

6.1.1 Blood Pressure and Circulating Levels of sICAM-1/sVCAM-1

The results of this dissertation provide further evidence that exposure to Pb, either prenatally or in early childhood, may have lasting effects on BP later in life. Exposure to Pb was characterized first by evaluating BPb levels, which had been measured every six months from birth through 12.5 years of age. BPb levels varied widely across the study early in life. We then measured concurrent PBb at age 25, by which time a significant reduction in BPb levels had occurred. Between ages 12 and 25 years, the mean BPb fell from 29.7 to 4.91 µg/dl in Mitrovica, and from 5.73 to 1.67 µg/dl in Prishtina. A major strength of this study is its exceptionally well-documented history of exposure prenatally and through early childhood.

The unadjusted regression coefficient for systolic blood pressure (sBP) was 1.47 mmHg (95 % CL 0.64-2.29) for each log unit increase in BPb. After adjusting for potentially confounding variables (BMI, smoking, education, gender, ethnicity), the regression coefficient for sBP remained statistically significant at 1.04 mmHg (95 % CL 0.09, 1.85 per log unit increase in BPb). The findings differed for diastolic blood pressure (dBP), where the unadjusted regression coefficient for sBP was 0.57 mmHg (95 % CL 0.14-1.00) for each log unit increase in BPb. After adjusting for potentially confounding variables, the regression coefficient was no longer statistically significant at 0.35 mmHg (95 % CL -0.13, 0.83 per log unit increase in BPb). The findings in this study are in agreement with a large number of studies of wide range of populations that report an increase in BP between 1-3 mmHg per log unit increase in BPb [Sharp et al. 1987; Pocock et al., 1988; Hertz-Picciotto and Croft, 1993; Nawrot et al. 2002; Schwartz, et al., 1995; Staessen et al., 1994, 1996; U.S. EPA 2006]. In addition, our study is also in agreement with findings that included members of this study's cohort at 5.5 years of age

(Factor-Litvak et al., 1996) and with findings in a few studies in children (Gump et al., 2005; Zhang et al., 2012).

We also examined the associations between sBP and the total area under the BPb vs. age curve (AUC) for post-natal periods of birth to age 2, 2-4, 4-7, and 7-12 years old. In this manner we attempted to determine if there is a critical time period of Pb exposure with regard to its impact on current sBP. The results did not reveal differences in the associations between BPb on sBP across these age windows.

Associations between BPb and circulating levels of sICAM-1 and sVCAM-1 were also examined. The unadjusted regression coefficient for sICAM-1 was 4.76 ng/ml (95 % CL 1.14, 8.38) for each µg/dL increase in BPb. After adjusting for potentially confounding variables, the regression coefficient was also not statistically significant at 3.37 ng/ml (95 % CL -0.42, 7.19). The unadjusted regression coefficient for sVCAM-1 was 12.92 ng/ml (95 % CL -1.32, 27.17). After adjustment, the regression coefficient was not significant at 10.37 ng/ml (95 % CL -4.49, 25.19) for each µg/dL increase in BPb.

Lastly, we also examined the associations between sICAM-1/sVCAM-1 and the total area under the BPb vs. age curve (AUC) for post-natal periods from birth to age 2, 2-4, 4-7, and 7-12 years old. In this manner we attempted to determine if there is a critical time period of Pb exposure with regard to its impact on current levels of sICAM-1/sVCAM-1. The results did not reveal differences in the associations between BPb on sICAM-1/sVCAM-1 across these age windows.

In summary, we found a statistically significant association between BPb and sBP, and a marginally significant association between BPb and dBP, which is consistent with a multitude of previously referenced studies and meta-analyses. These results provide further evidence that

recent circulating dose, as estimated by BPb, or as estimated by lifetime cumulative exposure, is associated with slight increase in sBP. Furthermore, we detected a suggestive relationship between BPb and levels of circulating sICAM-1 and sVCAM-1. The findings support the hypothesis that the exposure to Pb either prenatally or in early childhood poses a risk for elevated BP later in life.

6.1.2. EPO Production

Our results show a positive association between BPb and serum-EPO levels with a significantly more pronounced slope in the regression line for those participants with relatively low Hgb levels. A simple linear regression model relating log BPb to log serum EPO did not find a significant relationship between the two variables ($\beta = 0.05$; 95% CI -0.09, 0.20). However, when adjusted for Hgb as a continuous variable, the relationship became significant ($\beta = 0.19$; 95% CI 0.06, 0.31). The subsequent introduction of an interaction term between BPb and Hgb strengthened the relationship between log BPb and log serum EPO ($\beta = 0.77$; 95% CI 0.19, 1.36). In addition, significant positive associations were found for cumulative Pb exposure between birth and age 2, ages 2- 4, ages 4-7 and EPO. The estimated regression coefficients declined in magnitude as the children aged. However, no significant associations between cumulative lead exposure and EPO were found at age 7-12, but the sign of the estimated beta changed to negative. Overall, these results resemble the findings reported in this cohort earlier in life.

6.2 Study Challenges and Limitations

This thesis study relied on collection of the biological sample (blood) for laboratory analysis, blood pressure measurements, and primary data, all requiring extensive human subject approvals. The process included not only the approvals by Columbia University's institutional review board (IRB-AAAD8787), but also setting-up a similar structure in Kosovo and requesting their review and approval. In addition, we established an ongoing collaboration with this institution as well as specific departments within the Kosovo government (Ministry of Health) and the clinical center in Prishtina. After the IRB's approvals were secured, the major challenge was to locate and contact members of the original study cohort in order to request their participation. We utilized various methods to initiate the contact with the study participants, including use of social media ("Facebook"). The challenges also included: organizing and securing space and laboratory facilities for the sample collection, conduction interviews of the study participants as well as, funding for participant travel and meal expenses. Notably, there were funding limitations, as we did not have NIH support for this work.

6.3 Response Rate

While we would have preferred a larger number of study participants with better representation of the former cohort, we were able to get about 36.5% of the study population that was last evaluated in 1998 (at an average age of 12.5). It is important to note that the study participants were more likely to reside in Mitrovica and were more likely to be Albanian. In Mitrovica, 68% were Albanian, which is close to the make-up of the original cohort, where 61 % were from the Albanian population and the remainder was either Serbian or "other" populations. However, in Prishtina, 95% of the current study participants were Albanian. The current study

sample included 47.5 % males; this is slightly lower than the cohort at 5.5 years of age, when 54% were males.   In Prishtina, 80% of participants had a college degree and 76% were employed; in Mitrovica these proportions were 49% and 47%, respectively. Overall, although the current cohort is smaller (N-101) than during the last evaluation (N=280), the data analysis showed that Pb-exposed town's biomarkers were representative of the larger 1998 study sample.

6.4 Study Design: Strengths and Limitations

One of the main strengths of this study is that it followed a longitudinal design which allowed us to utilize several observations of the study participant over a period of time. The benefit of a longitudinal study is that it makes it possible to detect developments or changes in the characteristics of the target population at both the group and the individual level. Longitudinal studies extend beyond a single moment in time, and as a result, they can establish sequences of events.   Our study involved a wide range of BPbs measured early in life, albeit less of a range at age 25, and made possible the analysis of early life exposure on outcomes later in life. In addition, we had a range of Hgb levels measured in early life and BP measurements at an early age (5.5 years old). Lastly,   the sample used in this study has few adult risk factors for high BP, i.e. over 70% had BMIs between 20 and 24.9 and most were non - or "light" smokers (smoking a few to < 20 cigarettes a day). Overall, we can say that the significant correlations between measured points of exposure and outcomes reported in this study provide reasonably reliable tools that can predict the impact of Pb later in life.

Some of the study limitations include the fact that we only followed a select ~20% of the original full sample; however, there is no reason to assume that the biological relationships

would differ between those followed and those not followed. Another limitation is the 12 ½ year gap in the BPb measurements. Also, limitation pertains to the single measure of CVD risk markers. Finally, our analysis is based on a select population residing in Kosovo and the results may not be generalizable to other populations.

6.5 Public Health Implications and Future Directions

Although exposure to environmental Pb has been reduced precipitously in most of the developed world, exposure to Pb remains a significant threat to health in much of the developing world. BPb levels as low as 5 µg/dL can impair mental and physical development, especially in young children. Exposure to Pb can negatively impact heme biosynthesis, the nervous system, the kidneys, cardiovascular, hepatic, endocrinal and gastrointestinal systems (EPA, 2012; ATSDR, 2007; WHO, 2001). The most deleterious effects of Pb are on kidney function, and the central nervous system (ATSDR, 2007; Bellinger et al., 1995). In the last few decades, the effects of environmental Pb exposure have been examined in relation to human health. These evaluations involved populations with extremely high exposure levels (occupational or "smelter town" studies), as well as those with relatively low level exposures (population studies) discussed in the previous chapters. Studies have reported that BPb levels once deemed as "safe levels" (< 10 ug/dL), Pb can pose a significant threat to the human health.

With regard to the effect of Pb on the endocrine function of the kidneys, the study results, along with previously reported findings on this cohort, suggest that a dramatic reduction of Pb exposure may allow for a reversal of the impact that the prolonged Pb exposure may have on EPO production. However, even though the serum EPO concentrations responded appropriately

as a function of Hgb concentration, we report that they also increased inappropriately as concurrent BPb increased.   This study has provided additional evidence or a reminder that first, a prolonged exposure to Pb may play a significant role in disrupting the endocrine system function; and second, a significant continuous reduction of exposure to Pb may allow for a reversal of the impact Pb may have on EPO synthesis.

REFERENCES (CHAPTER VI)

Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead. Atlanta, GA: US Department of Health and Human Services, Public Health Service, 2007.

Bellinger, D, Dietrich, K. 1995. Low-level lead exposure and cognitive function in children, Pediatr. Ann. 23 600–605.

Factor-Litvak P, Graziano JH, Kline J, Popovac D,Mehmeti A, Ahmedi G, Shrout P, Murphy MJ, Gashi E,Haxihu R, et al. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. Int J Epidemiology. 20:722-728.

Factor-Litvak, P, Kline, J, Popovac, D, Hadzialjevic, S, Lekic, V, Preteni-Redjepi, E, Capuni-Paracka, S, Slavkovich, V, Graziano, JH. 1996. Blood lead and blood pressure in young children. Epidemiology. 7: 633-637.

Graziano, JH, Slavkovic, V, Factor-Litvak, P, et al. 1991.Depressed serum erythropoietin in pregnant women with elevated blood lead. Archives of Environmental Health 46: 347-350.

Gump, BB; Stewart, P; Reihman, J; Lonky, E; Darvill, T; Matthews, KA; Parsons, PJ. 2005. Prenatal and early childhood blood lead levels and cardiovascular functioning in 9 1/2 year old children. Neurotoxicol Teratol 27: 655-665.

Hertz-Picciotto I, Croft J. 1993. Review of the relation between blood lead and blood pressure. Epidemiology Rev. 15: 352–73.

Murphy, MJ, Graziano, JH, Popovac, D, Kline, J, Mehmedi, A, Factor-Litvak, P, Ahmedi, G, Shrout, P, Rajovic, B, Nenezic, DU, Stein, ZA. 1990. Past Pregnancy Outcomes among Women Living in the Vicinity of a Lead Smelter in Kosovo, Yugoslavia. Amer J Pub Health 80: 33-35.

Nawrot TS, L Thijs1, EM Den Hond, HA Roels and JA Staessen. 2002. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis Journal of Human Hypertension. 16, 123–131.

Pocock,S., Shaper, A., Ashby, D., Delves, H., and Clayton B. 1988. The relationship between blood lead, blood pressure, stroke, and heart attacks in middle-aged British men. Environ Health Perspectives. 78: 23–30.

Schwartz J. 1995. Lead, blood pressure, and cardiovascular disease in men. Arch Environ Health 50:31–37.

Sharp DS, Becker CE, Smith AH. 1987. Chronic low-level lead exposure: its role in the pathogenesis of hypertension. Med Toxicol. 2:210–232.

Staessen, J. A.; Bulpitt, C. J.; Fagard, R.; Lauwerys, R. R.; Roels, H.; Thijs, L.; Amery, A. 1994. Hypertension caused by low-level lead exposure: myth or fact? J. Cardiovasc. Risk 1: 87-97.

Staessen, J. A, Roels, H, Fagard, R. 1996a. Lead exposure and conventional and ambulatory blood pressure: a prospective population study. JAMA J. Am. Med. Assoc. 275: 1563-1570.

U.S. EPA. Environmental Protection Agency. 2006. Air Quality Criteria for Lead (Final).U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC, USA.

U.S. EPA. Integrated Science Assessment for Lead. 2012. EPA/600/R-10/075B.

WHO Regional Office for Europe, Copenhagen, Denmark. 2001. Chapter 6.7 Lead Air Quality Guidelines - Second Edition Wu, T., ' Shen, C., Ko, K., Guu, C., Gao, H., Lai, J., Chen C., Chng, P. 199). Occupational lead exposure and blood pressure. International Journal of Epidemiology**.** 25, No. 4.

Zhang, A; Hu, H; Sánchez, BN; Ettinger, AS; Park, SK; Cantonwine, D; Schnaas, L; Wright, RO; Lamadrid-Figueroa, H; Tellez-Rojo, MM. 2012. Association between Prenatal Lead Exposure and Blood Pressure in Female Offspring. Environ Health Perspectives 120 (3) 445-450.

APPENDIX (TABLES AND FIGURE NOT USED IN THE PUBLICATION)
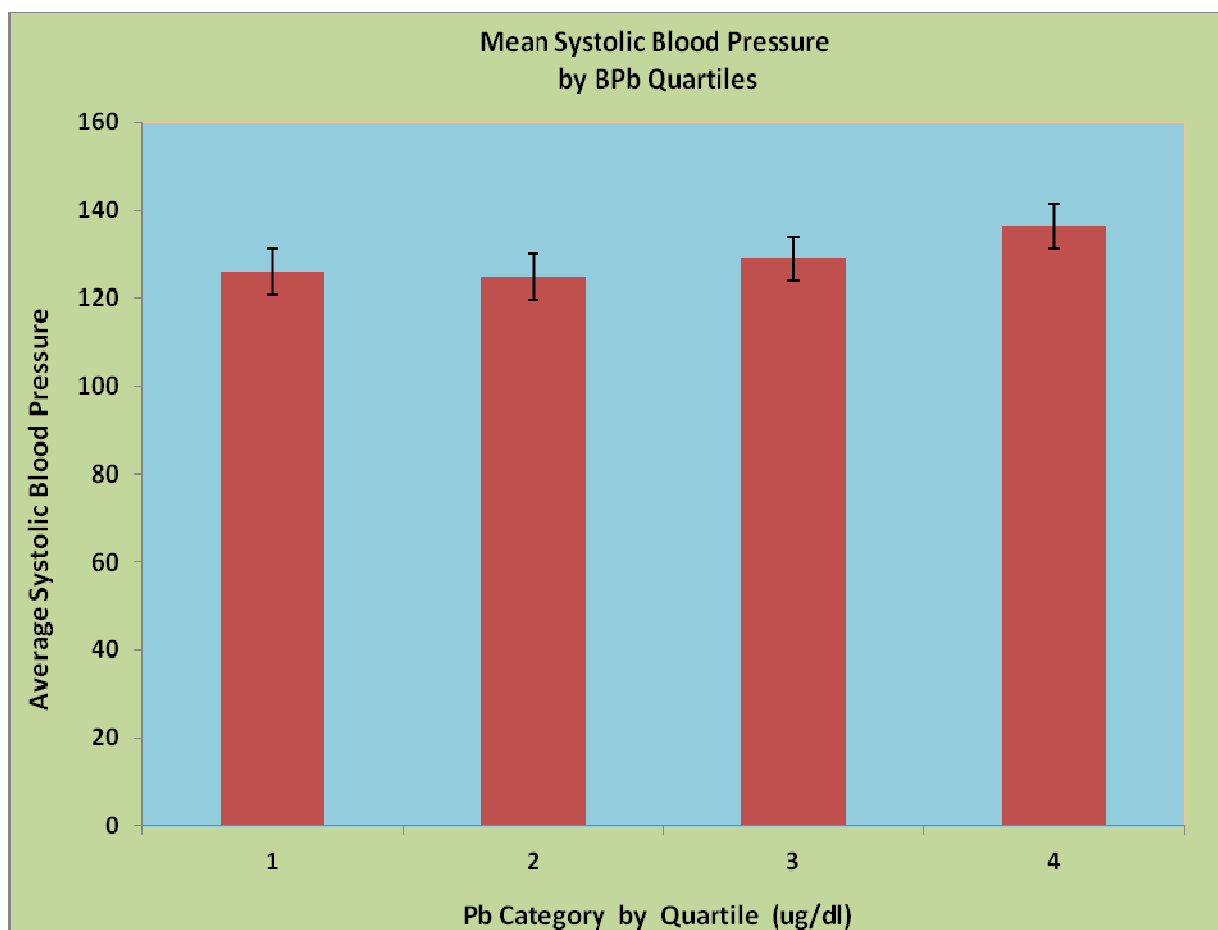
Appendix Figure 16 – Relationship between sBP and mean BPb increments by BPb quartiles

Appendix Table 14: Apparent Half-life of Elimination of Pb in Mitrovica and Prishtina (in years)

	Prishtina	Mitrovica	P-Value	95% CI
Mean	7.4	4.9	0.0001	-3.303, -1.069
SD	2.3	1.4		
SEM	0.5	0.15		
Max	13	9		
Min	3	3.2		
Range	10	6		

Appendix Table 15: Historical and Current Blood Lead Levels

	Prishtina (N=21)			Mitrovica (N=80)		
Age at Pb Measure	**Mean**	**SD**	**P-Value**	**Mean**	**SD**	**P-value**
Mid Pregnancy		5.73	3.36	21.01	7.63	<0.0001
Cord	6.14	3.51		22.83	7.92	<0.0001
6 months	5.87	3.31		24.63	8.49	<0.0001
12 months	7.38	3.31		30.25	10.00	<0.0001
18 months	7.55	2.55		35.58	10.92	<0.0001
24 months	8.74	5.36		39.63	12.42	<0.0001
30 months	9.36	2.57		39.34	12.91	<0.0001
36 months	10.25	3.12		42.33	14.00	<0.0001
42 months	9.76	2.62		41.11	12.17	<0.0001
48 months	9.69	3.33		41.84	11.50	<0.0001
54 months	8.64	3.20		40.00	11.06	<0.0001
60 months	8.51	2.49		37.78	9.37	<0.0001
66 months	9.11	2.98		38.06	10.08	<0.0001
72 months	8.30	2.41		36.02	9.93	<0.0001
78 months	8.61	2.73		35.47	10.23	<0.0001
84 months	9.62	7.58		31.48	10.07	<0.0001
90 months	9.60	---		31.37	10.21	----*
9.5 years	7.39	3.89		29.29	9.20	<0.0001
10 years	7.07	3.44		29.07	8.79	<0.0001
10.5 years	7.31	2.76		31.60	10.77	<0.0001
11 years	6.64	2.40		30.00	9.22	<0.0001
11.5 years	5.50	1.79		31.17	9.42	<0.0001
12 years	6.61	3.02		29.81	8.73	<0.0001
12.5 years	5.73	1.42		29.70	7.10	<0.0001
25 years	1.67	0.67		4.91	3.26	<0.0001

Notes: *No estimate provided due to low sample size.

Appendix Table 16: Regression Coefficients Relating Selected Characteristics to Systolic Blood Pressure

	Systolic Blood Pressure				Diastolic Blood Pressure			
	β	**95% CL**	**N**	**R^2**	**β**	**95% CL**	**N**	**R^2**
Intercept	105.7	95.74, 115.67	95	0.2824	69.97	95.74-115.67	95	0.2265
BPb	1.04	0.25, 1.82			0.33	-0.09, 0.74		
Current Smoking	1.03	-4.24, 6.31			0.12	-4.24, 6.31		
BMI	12.82	7.38, 18.26			6.79	7.38, 18.26		

Appendix Table 17: Linear Regression Models for Lead Exposure at Various Periods of Development and Mean Systolic Blood Pressure at Age 25

	β	95% CL	R-Square	P-Value
Univariate				
Period of Exposure				
0-2 years	0.007	-0.00, 0.02	0.02	0.14
2-4 years	0.007	-0.00, 0.01	0.03	0.07
4-7 years	0.006	0.00, 0.01	0.04	0.04
7-12.5 years	0.006	0.00, 0.01	0.06	0.02
Multivariable*				
Period of Exposure				
0-2 years	0.007	-0.003, 0.018	0.03	0.16
2-4 years	0.006	-0.001, 0.014	0.04	0.11
4-7 years	0.005	-0.00, 0.01	0.05	0.05
7-12.5 years	0.005	0.001, 0.01	0.05	0.03

adjusted for ethnicity and education

Appendix Table 18: Linear Regression Models for Lead Exposure at Various Periods of Development and sVCAM-1 and s-ICAM-1 at Age 25

	sVCAM-1				sICAM-1			
	β	95% CL	R^2	P-Value	β	95% CL	R^2	P-Value
Univariate					**Univariate**			
Period of Exposure								
0-2 years	0.09	-0.08, 0.25	0.011	0.31	0.03	-0.02, 0.07	0.016	0.21
2-4 years	0.06	-0.06, 0.18	0.009	0.34	0.03	-0.01, 0.06	0.035	0.11
4-7 years	0.04	-0.06, 0.13	0.006	0.43	0.02	-0.006, 0.04	0.022	0.15
7-12.5 years	0.02	-0.06, 0.10	0.003	0.61	0.02	-0.005, 0.04	0.025	0.14
Multivariable*					**Multivariable***			
Period of Exposure								
0-2 years	0.05	-0.13, 0.22	0.0001	0.60	0.03	-0.02, 0.07	0.016	0.21
2-4 years	0.04	-0.09, 0.16	0.0009	0.56	0.02	-0.01, 0.05	0.04	0.26
4-7 years	0.027	-0.07, 0.12	0.0006	0.57	0.014	-0.01, 0.04	0.04	0.25
7-12.5 years	0.013	-0.068, 0.09	0.0033	0.76	0.012	-0.009, 0.03	0.04	0.25

adjusted for ethnicity and education

Appendix Table 19: Linear Regression Models for Lead Exposure at Various Periods of Development and EPO at Age 25

	β	95% CL	P-Value	R-Square
Univariate				
Period of Exposure				
0-2 years	0.00024	-0.000094, 0.00057	0.16	0.021
2-4 years	0.00028	0.000022, 0.00054	0.03	0.046
4-7 years	0.000129	-0.000068, 0.00032	0.2	0.017
7-12 years	-0.000062	-0.00023, 0.000107	0.47	0.006
Multivariable*				
Period of Exposure				
0-2 years	0.00043	0.000081, 0.00077	0.02	0.08
2-4 years	0.00036	0.00010, 0.0062	0.006	0.086
4-7 years	0.00016	-0.000035, 0.00035	0.11	0.04
7-12 years	-0.00003	-0.00020, 0.00013	0.66	0.025

adjusted for ethnicity and gender